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The publication of the *Journal*, together with its predecessors, has been continuous since 1901. Its predecessors are *Contributions to Canadian Biology* published from 1901 to 1921, *Contributions to Canadian Biology and Fisheries* from 1922 to 1936, and the *Journal of the Biological Board of Canada* from 1936 to 1937.

This *Journal* is published in monthly volumes of twelve issues. This issue (Vol. 25, No. 2) was published February 16, 1968.

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All Fisheries Research Board of Canada publications are for sale by the Queen's Printer, Ottawa. Remittances must be in advance, payable in Canadian funds to the order of the Receiver General of Canada. Subscriptions rates to the *Journal* are: Canada, United States, Mexico, $7.50, single issue $1.00; to other countries, $8.75 and $1.25.

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Effect of Pond Formation on Catches of Brook Trout from a Coastal Stream System[1]

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ABSTRACT

An artificial 5-acre (2.0-ha) pond formed on Ellerslie Brook, Prince Edward Island, initially resulted in an increased fishing effort and yield of brook trout to anglers from the stream system — estuary, pond, and stream. The increased effort and catch were not sustained. After 4 years, annual effort and yield of trout by number from the system were not significantly different from the pre-pond period. Length of angled trout and yield by weight declined significantly. Sea-running trout, prized by anglers, were largely eliminated. Only when planted into the estuary did hatchery-reared trout make good contributions to the anglers' catches.

INTRODUCTION

Ponds are being formed on small coastal streams of the Maritime provinces of Canada to improve yield of trout (*Salvelinus fontinalis* (Mitchill)) to anglers. Brook trout run to salt water from these coastal streams (White, 1940, 1941, 1942; Wilder, 1952; Smith and Saunders, 1958). Accordingly, it is important in the management of the brook trout fishery to learn what effects pond formation has upon the movements, growth, and exploitation by anglers of brook trout from a stream system — estuary, pond, and stream.

A study has been made at Ellerslie Brook, Prince Edward Island, to assess the effects of pond formation upon movements and population of brook trout, and upon the yield of this species to anglers from estuary, pond, and stream. The movements of trout between and within fresh- and salt water before and after pond formation have been described (Smith and Saunders, 1958, 1967). Analyses of angling records obtained from annual creel censuses on the Ellerslie Brook System from 1947 to 1965, before and after pond formation, are presented herein.

ELLERSLIE BROOK SYSTEM

A description of Ellerslie Brook and estuary has been given by Smith and Saunders (1958). The freshwater portion of the Ellerslie Brook System is a spring-fed stream, about 6 miles (1 mile = 1.609 km) in length, and tributary to Malpeque Bay on the north shore of Prince Edward Island (lat 46°35′N, long 63°57′W). For the purpose of this study, the seaward limit of Ellerslie estuary was arbitrarily fixed at 2¼ miles from the mouth of Ellerslie Brook, where the estuary widens out into Malpeque Bay. From the recapture of

[1]Received for publication August 10, 1967.

J. Fish. Res. Bd. Canada, 25(2): 209–238, 1968.
Printed in Canada.

tagged trout in smelt gill-nets, it appears that the main body of Ellerslie trout in the estuary remains above this designated boundary at least in autumn and winter.

METHODS

(*a*) *Trapping and counting fish.*— A counting fence (estuarial) was operated at the head of tide from 1946 onward (Smith and Saunders, 1956). A second counting fence (stream) was installed 650 yards (1 yard = 0.9144 m) above the estuarial fence in 1950. Two traps were incorporated in the fences to capture fish moving up- and downstream. After pond formation, the estuarial fence was about 30 yards below the dam, and the stream fence just above the head of the pond. The estuarial fence was entirely downstream from a pool formed below the dam. Both fences were operated year-round; the traps were cleared daily.

(*b*) *Pond formation.*— A 5-acre (2.0-ha) pond was formed immediately above the head of tide in the autumn of 1952 (Fig. 1). The pond flooded about 625 yards of the lower part of the brook. It has a maximum depth of 10 ft (1 ft = 0.3048 m). Smith and Saunders (1967) have described the pond and certain of its limnological characteristics.

(*c*) *Pool formation.*— A consequence of forming the pond was development of a pool below the apron of the dam. The pool was tidal, had an approximate area of 4000 ft^2 (1 ft^2 = 0.0929 m^2) with a maximum depth around 5 ft at low tide.

(*d*) *Stream improvement.*— Low dams, deflectors, and cover were constructed in a 450-yard section of Hayes Brook, a small tributary to Ellerslie Brook, in 1959 (Saunders and Smith, 1962). In 1961 similar stream improvements were applied more extensively on the main stream, and affected 2000 yards of the brook upstream from the head of the pond.

(*e*) *Transfer of trout from estuary to pond.*— In the years 1953–55, trout that moved up from the estuary and were captured at the estuarial fence were tagged and transferred to the pond. The purpose of the transfer was to assess effects of the pond upon later movements of these trout.

(*f*) *Closure of pool below dam to angling.*— The pool was closed to angling from 1953 to 1955. The restriction was to aid in the assessment of further movements of trout that came over the dam, without reduction of their numbers from angling in the pool. Angling was permitted in the pool in 1956 and thereafter.

(*g*) *Stocking with hatchery-reared trout.*— Hatchery-reared progeny from sea-running and non-sea-running parent brook trout were introduced into Ellerslie Brook to learn if the former had a greater tendency to run to salt water (Smith and Saunders, 1958). Hatchery-reared trout were also introduced into the estuary to compensate for the loss of naturally reared stock from the stream after pond formation (Saunders and Smith, 1964). Equal numbers were planted in the estuary and stream in 1965 to compare survival.

(*h*) *Tagging.*— Trout taken in the traps at the two fences, or captured elsewhere in the system by electrofishing during population studies, were jaw-tagged (Smith, 1957; Smith and Saunders, 1958).

(*i*) *Creel census.*— A creel census was maintained annually from 1947 through 1965. There are records of 13,077 rod-days of fishing on the system during the study period. Of this number, 5790 (44%) were applied to fishing after 5 PM, and a much smaller number, 83, before 8 AM. It was important that the census-taker was on duty during the evening.

Most records were obtained by the fishermen submitting their catches for examination. Records for boat fishermen on the pond were virtually complete since the boats were under control of the census-taker. Some anglers were contacted after they had left the stream. Over the years such anglers accounted for less than 1% of the total number of rod-days. The fishing area of the Ellerslie System was small, and the angling record is considered nearly complete.

The creel census data, entered on cards in the field, included number of rods, number of trout caught, method of fishing, usually fork length of each fish in centimetres, and, from 1953 through 1965, weight of each fish in grams. Five areas of angling were designated: estuary, pool below dam, stream area flooded by pond, pond, and stream above pond; in addition the pond

was divided into seven fishing subareas. Cards were filled out for anglers whether trout were captured or not. In some years a few juvenile Atlantic salmon were also taken by anglers (Saunders, 1960). There were 209 trout out of 31,223 angled during the study for which the fishing effort was not recorded. Of 22,126 trout captured during the period 1953–65 when weights were taken, 2568 were not weighed. The weight of these trout, as well as those taken from 1947 through 1952, was estimated from the length–weight relationship: $W = 0.009515L^{3.097}$ (W = weight in grams and L = fork length in centimetres). The relationship was established for Prince Edward Island brook trout in the length range 12.5–21.1 cm, which embraced most of the trout in Ellerslie Brook. Fork lengths of 28,985 trout were obtained.

To induce anglers to submit catches for examination, a small reward was paid for each recovered tag. Payment of rewards may have increased the fishing effort, but no evaluation of this opinion is possible since rewards were paid throughout the study.

FISHING REGULATIONS

The catch limit of brook trout for Prince Edward Island is 20 per day and there is no season limit. Until 1958, the angling season was from April 15 to September 15, but in that year it was extended to September 30. Prior to 1955 the minimum size of brook trout that could be retained by an angler was 6 inches (15.2 cm) (total length), but for 1955 and for later years there has been no size limit. All angled trout were recorded whether or not they were of legal size.

METHODS OF FISHING

Most effort was in still-fishing with bait, largely earthworms. There was little fly-fishing or spinning in the brook above the pond since this area was well wooded. The effort expended by the three methods of fishing is shown in Table I.

TABLE I. Fishing effort and catch according to methods of angling on the Ellerslie System.

	Still (bait)	Fly	Spin
Per cent of rods	87.1	11.2	1.7
Per cent of rod-hours	89.5	10.2	0.3
Per cent of catch	91.9	7.2	0.9
Catch per rod	1.7	1.6	0.3
Catch per rod-hour	1.3	2.0	0.4

ANGLING STATISTICS

Monthly statistics of fishing effort as rod-hours, number of trout caught, and catch per rod-hour are presented separately in Tables II–VI for the pond, pool below the dam, stream area flooded by pond, estuary, and stream area above the pond. All the angling records are combined in Table VII.

POND

Accumulation of trout in the pond.— The pond delayed further upstream and downstream movements of most of the trout that entered it (Smith and

Saunders, 1967). An accumulation of trout in the pond resulted. During the early years, fish in the pond originated (*a*) from delay of trout which moved down from the stream, particularly those in the prominent runs in autumn and early winter; (*b*) from delay of trout transferred to the pond from the estuarial fence, 1953–55; and (*c*) from retention of those trout which were in the stream area flooded in forming the pond in 1952. However, transfer of trout to the pond from the estuarial fence was discontinued in 1956. Also by 1956 the trout that had been in the stream area flooded by the pond were largely gone from the population as a result of angling and natural mortalities. Brook trout are short-lived. Few trout survived to their 5th year of age (age 4+) at Ellerslie Brook (Smith and Saunders, 1958). Accordingly, from 1957 through 1965, trout in the pond were entirely those that annually moved down from the stream. Trout that went over the dam, which was not screened to prevent such movements, were lost to the pond.

Catch from pond.— In 1953, the novelty of a new pond, with boats available to fish from, and ready accessibility of the pond attracted more anglers (Table II). However, the good fishing resulting from the accumulation of trout was the dominant factor that stimulated a greater angling effort on the pond alone than had been experienced on the entire Ellerslie System during any of the previous 6 years (Tables II and VII). Most of the effort, as rod-hours (93%), and the catch (93%) from the Ellerslie System in 1953 were on the pond. With continuing favourable catches from the pond in early 1954, still greater effort was applied and a higher catch realized in the latter year (Table II). In 1954, 81% of the fishing effort and 79% of the catch were on the pond. The peak of effort and catch for the pond occurred in 1954. Thereafter, effort and catch on the pond declined. From 1957 to 1965, when accumulation of trout in the pond had declined, the average annual effort was 380 (259–561) rod-hr yielding an annual average catch of 387 (334–588) trout as compared with 2095 rod-hr and 2271 trout in 1954. The catch from the pond from 1953 to 1965 was 47% of the total catch for the Ellerslie System. The yield was 70% of the total catch during early years of the pond's existence (1953–56). During the intermediate period, 1957–61, the yield from the pond was 37% of the total catch from the system, and in the last 4 years of the study period (1962–65) it fell to 28% (Tables II and VII).

Boat vs. shore angling.— From 1953 through 1963, 1085 rods with 1622 hr fishing on the pond from boats took 1532 trout at an average rate of 0.9 trout per rod-hour. During the same period, 4778 rods fished the pond from the shore for 6630 hr, catching 7922 trout at an average rate of 1.2 fish per rod-hour. (The number of rods and anglers was not equal since some anglers visited Ellerslie more than once.)

Areas of capture in pond.— The pond was roughly divided into seven areas, and so far as possible the catches were recorded for each area (Fig. 1). Neither effort nor catch was evenly distributed over the pond. Most trout were taken in or close to the old stream bed, and for each area this was the greatest depth of water. Angling in two areas (C and D) accounted for 57%

TABLE II. Angling statistics for Ellerslie pond. Numbers of planted hatchery-reared trout given in parentheses but included in monthly and seasonal totals.

	No. of trout caught							Fishing effort as rod-hr							
	Apr.	May	June	July	Aug.	Sept.	Season	Apr.	May	June	July	Aug.	Sept.	Season	Per rod-hr
1953	691	534	186	59	213	132	1,815	363	400	135	67	195	74	1,234	1.5
1954	566	575	405	592	95	38	2,271	528	524	367	464	147	65	2,095	1.1
1955	360	257	261	89	104	19	1,090(4)	333	443	308	142	137	26	1,389	0.8
1956	364	210	122	40	21	44	801(90)	265	215	127	87	29	45	768	1.1
1957	7	220	81	96	127	57	588(18)	55	169	103	79	124	31	561	1.0
1958	79	45	32	75	64	14	309(3)	109	73	37	81	56	28	384	0.8
1959	498	41	7	46	47	49	688	240	74	29	39	43	46	471	1.5
1960	307	22	22	24	30	4	409	274	40	38	48	17	3	420	1.0
1961	96	84	26	113	3	12	334	46	71	36	82	10	14	259	1.3
1962	84	144	94	142	36	16	516	39	92	77	98	40	9	355	1.5
1963	508	33	19	26	15	32	633	184	41	15	46	17	23	326	1.9
1964	313	53	63	15	12	23	479	118	61	72	49	13	23	336	1.4
1965	101	166	79	52	20	6	424	52	153	35	46	3	16	305	1.4
1953–65	3,974	2,384	1,397	1,369	787	446	10,357(115)	2,606	2,356	1,379	1,328	851	403	8,903	1.2
Per rod-hr	1.5	1.0	1.0	1.0	0.9	1.1	1.2								

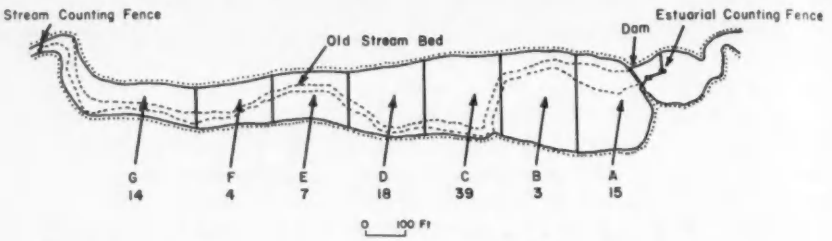

FIG. 1. Ellerslie Pond showing percentage of catch of trout made in various areas. Original stream bed shown by dotted outline through pond area.

of the total catch from the pond over the study period. These areas were where the old stream bed came close to the pond shore, and they were heavily fished from the shore. For the first half (April–June) of the angling seasons, 14% of the total annual catches from the pond was taken from the lower sections (A and B). However, in the second half of the seasons, 28% came from these lower sections. With the advance of summer, the trout tended to congregate in the cooler, deeper waters of the pond, in keeping with the known movements of brook trout into a preferred range of water temperatures (Fry, 1947; Smith and Saunders, 1958).

Pool below Dam

As with the pond, the pool delayed farther movements (Smith and Saunders, 1967). The pool acted as a fish-trap. Thus, there was an accumulation of trout in the pool from those dropping over the dam from the pond, as well as from those moving in from the estuary. The pool yielded good catches to the anglers and continued to do so through the study period (Table III). Only minor changes in the physical characteristics of the pool occurred from year to year. Accordingly, it may be judged that the pool did not deteriorate in its holding capacity for trout with advance of years.

During the years (1956–65) that the pool was fished, it yielded 33% of the total number of trout taken from the Ellerslie System (Tables III and VII). The pool was small compared with the areas of the pond and stream above and of the estuary below. That about one-third of the catch for the system came from such a relatively small fishing area emphasizes the role of the pool in holding trout where they were highly vulnerable to angling pressure.

Flooded Area of Stream

The 650 yards of the lower part of the brook later flooded by the pond had pools which were good trout habitats and were heavily fished. From 1947 through 1952, angling in this section accounted for 45% of the effort as rod-hours and 44% of the total catch from the system (Tables IV and VII).

Effort and catch from the stream area later flooded were significantly lower ($p < 0.01$) than from the pond during the early years of its formation (1953–56) (Tables II and IV). (Significance of difference was determined

TABLE III. Angling statistics for pool below Ellerslie pond dam. Numbers of planted hatchery-reared trout given in parentheses but included in monthly and seasonal totals.

	No. of trout caught							Fishing effort as rod-hr							
	Apr.	May	June	July	Aug.	Sept.	Season	Apr.	May	June	July	Aug.	Sept.	Season	Per rod-hr
1956	322	187	153	13	6	21	702(31)	200	153	163	38	17	17	588	1.2
1957	73	587	64	19	11	0	754(14)	190	277	75	23	16	0	581	1.3
1958	68	63	31	52	0	12	226(2)	177	99	32	47	1	13	369	0.6
1959	65	59	100	17	18	3	262	87	67	69	27	17	8	275	1.0
1960	322	81	26	9	5	13	456	194	56	17	9	6	10	292	1.6
1961	3	95	30	15	5	9	157	8	84	20	21	1	6	140	1.1
1962	240	248	248	51	7	6	800(514)	90	125	94	50	7	5	371	2.2
1963	1	544	67	13	0	18	643(352)	11	266	35	13	1	10	336	1.9
1964	54	283	80	23	2	7	449(110)	14	169	47	23	3	6	262	1.7
1965	196	281	172	9	3	1	662(124)	85	134	79	10	4	1	313	2.1
1956–65	1,344	2,428	971	221	57	90	5,111(1,147)	1,056	1,430	631	261	73	76	3,527	1.4
Per rod-hr	1.3	1.7	1.5	0.8	0.8	1.2	1.4								

TABLE IV. Angling statistics for stream area flooded by pond. Numbers of planted hatchery-reared trout given in parentheses but included in monthly and seasonal totals.

	No. of trout caught							Fishing effort as rod-hr							Per rod-hr
	Apr.	May	June	July	Aug.	Sept.	Season	Apr.	May	June	July	Aug.	Sept.	Season	
1947	29	394	95	25	12	3	558	57	211	74	22	6	1	371	1.5
1948	30	260	110	14	27	0	441	35	163	76	14	10	0	298	1.5
1949	352	452	173	28	10	0	1,015	202	204	120	55	5	0	586	1.7
1950	68	322	65	55	8	3	521(106)	63	191	52	29	7	3	345	1.5
1951	274	134	90	74	47	51	670(35)	154	84	36	36	18	16	344	1.9
1952	122	324	97	99	76	2	720(1)	61	107	39	43	52	1	303	2.4
1947–52	875	1,886	630	295	180	59	3,925(142)	572	960	397	199	98	21	2,247	1.8
Per rod-hr	1.5	2.0	1.6	1.5	1.8	2.8	1.8								

here and later by Students *t*-test.) Later, effort and catch on the pond declined, until in the years 1957–65 the average annual effort on the pond was only slightly higher and the average annual catch somewhat lower, although not significantly so ($p > 0.05$), than for the flooded stream section.

The pool below the dam was also a new habitat created by pond formation, replacing a small section of the lower part of the stream. It is appropriate, therefore, to combine the effort and catch for pond and pool below the dam when comparing the statistics for the lower 650-yard section of the stream before and after flooding. The pool was opened to fishing in 1956. In that year, and in 1957, the combined effort and catch for the pond and pool were maintained at levels appreciably higher than in the pre-pond stream area, as was the case for the pond alone in the period 1953–55 (Table VIII). During the next 8 years, 1958–65, the average annual effort as rod-hours on the pond and pool was lower than in the early years of their existence (1953–57), but still significantly above ($p < 0.01$) that previously applied to the flooded stream area. Probably because of availability of the estuarially planted hatchery-reared trout in the pool from 1962 to 1965, the fishing effort on the pool was well sustained (Table III). The average annual catch of native trout from pond and pool (1958–65) was also higher, but not significantly ($p > 0.05$), than that from the flooded stream (1947–52). It is apparent that the pond and the pool below the dam together stimulated a greater fishing effort than the stream area they replaced, and there resulted a significantly higher annual catch of native trout in the early but not late years after pond formation.

Angling in Estuary

Angling effort as rod-hours and catch in the estuary showed marked variation over the study period (Table V). Most angling was in pools well up the estuary near the head of tide. Most trout in these pools were transients, moving between fresh- and salt water. Their stay in the area was largely determined by frequency and extent of spates in the brook, temperature of water, and phase of the tides. Some were resident throughout the summer but in general their catchability was poor.

In 1953 effort and catch in the estuary were low because most anglers fished the new pond. The pond delayed the seaward movements of trout so that they suffered heavy angling and natural mortalities in the pond (Smith and Saunders, 1967). With advance of years, decreasing numbers of trout moved between fresh- and salt water. Thus, from 1961 to 1965 an average of only 42 native trout were caught in the estuary. The catch in 1962–65 was augmented by the hatchery-reared trout planted in the estuary (Table V).

Angling in the estuary accounted for 9% of the total effort as rod-hours and 10% of the total catch for the system.

Angling in the Stream above the Pond

As in the estuary, effort and catch for the stream above the pond were much reduced in 1953, when most of the fishing effort was applied to the new pond (Table VI). In 1954 and after, a greater number of anglers returned to

TABLE V. Angling statistics for Ellerslie estuary. Numbers of planted hatchery-reared trout given in parentheses but included in monthly and seasonal totals.

	No. of trout caught							Fishing effort as rod-hr							Per rod-hr
	Apr.	May	June	July	Aug.	Sept.	Season	Apr.	May	June	July	Aug.	Sept.	Season	
1947	0	0	35	1	7	1	44	1	0	11	2	3	1	18	2.3
1948	0	52	120	20	4	0	196	0	18	86	16	5	0	125	1.6
1949	14	191	87	1	0	0	293(1)	7	132	70	1	1	0	211	1.4
1950	239	179	10	1	0	0	429(35)	89	90	13	3	0	0	195	2.2
1951	150	12	13	0	1	0	176(6)	65	21	9	2	1	0	98	1.8
1952	47	62	47	23	0	0	179	28	31	10	8	0	0	77	2.3
1953	0	12	32	7	3	1	55	2	5	22	5	1	1	36	1.5
1954	15	201	60	5	0	0	281	19	142	47	6	0	0	214	1.3
1955	37	71	36	29	3	11	187	34	39	42	25	4	3	147	1.3
1956	11	77	90	76	5	0	259(12)	18	30	76	46	5	1	176	1.5
1957	0	19	21	12	0	5	57	18	12	14	4	2	1	51	1.1
1958	0	80	58	21	0	11	170(1)	7	66	29	18	3	7	130	1.3
1959	25	21	35	21	5	0	107	26	13	35	12	5	1	92	1.2
1960	7	42	71	9	0	0	129	17	21	28	11	2	0	79	1.6
1961	0	30	32	0	2	2	66	5	22	16	0	1	2	46	1.4
1962	1	16	16	14	7	2	56(41)	1	5	5	4	3	1	19	3.0
1963	0	71	135	21	1	0	228(189)	1	46	71	38	2	0	158	1.4
1964	0	28	12	8	0	2	50(9)	0	31	11	5	0	2	49	1.0
1965	0	40	35	4	0	0	79(29)	6	26	8	3	0	0	43	1.8
1947–65	546	1,204	945	273	38	35	3,041(323)	344	750	603	209	38	20	1,964	1.5
Per rod-hr	1.6	1.6	1.6	1.3	1.0	1.8	1.5								

TABLE VI. Angling statistics for Ellerslie Brook above pond level. Numbers of planted hatchery-reared trout given in parentheses but included in monthly and seasonal totals.

	No. of trout caught								Fishing effort as rod-hr							
	Apr.	May	June	July	Aug.	Sept.	Season		Apr.	May	June	July	Aug.	Sept.	Season	Per rod-hr
1947	17	229	51	185	28	23	533		55	128	52	110	21	13	379	1.4
1948	7	138	56	129	80	9	419		9	71	44	86	68	7	285	1.5
1949	170	199	172	107	33	12	693(1)		82	96	91	81	18	11	379	1.8
1950	88	252	88	79	69	48	624(72)		50	135	48	52	48	16	349	1.8
1951	202	86	74	116	62	60	600(20)		89	56	20	44	25	17	251	2.4
1952	184	219	104	147	80	0	734(6)		83	90	48	63	52	0	336	2.2
1953	26	14	4	16	17	13	90		18	9	2	12	9	1	51	2.0
1954	28	56	72	66	25	71	318		10	33	32	28	11	37	151	2.4
1955	53	80	95	44	67	45	384(2)		22	26	42	17	23	12	142	2.7
1956	0	2	19	44	133	67	265(8)		0	3	2	14	63	20	102	2.6
1957	0	152	51	20	4	42	269(7)		0	45	16	5	9	6	81	3.3
1958	0	72	115	78	60	195	520(2)		0	35	26	28	10	61	160	3.3
1959	27	13	54	19	40	0	153		11	6	27	27	29	0	100	1.5
1960	26	125	134	86	27	37	435		25	59	44	33	21	13	195	2.2
1961	0	31	18	68	18	39	174		0	19	11	42	16	12	100	1.7
1962	43	95	117	243	26	194	718		14	35	54	93	21	71	288	2.5
1963	1	84	155	69	59	158	526		2	48	76	52	32	56	266	2.0
1964	0	71	246	142	90	52	601		0	34	78	56	40	29	237	2.5
1965	14	94	257	78	38	43	524		3	57	108	35	25	18	246	2.1
1947–65	886	2,012	1,882	1,736	956	1,108	8,580	(118)	473	985	821	878	541	400	4,098	2.1
Per rod-hr	1.9	2.0	2.3	2.0	1.8	2.8	2.1									

the stream. However, effort and catch remained significantly below ($p > 0.05$) pre-pond levels until 1962. From 1962 to 1965, catch returned to pre-pond numbers, coincident with stream improvement. The average annual catch per rod-hour from the stream was significantly higher ($p < 0.05$) for post-pond years.

Ellerslie System

Angling statistics for the entire Ellerslie System are consolidated in Table VII. From 1947 through 1965 there was a total of 20,739 rod-hr of angling on the Ellerslie System. The resulting average annual catch was 1632 ± 467 (standard deviation), with a threefold range in number from 731 in 1961 to 2870 in 1954. If the 2 extreme years are excluded, 1954 and 1961, the range was narrowed from 1056 in 1949 to 2090 in 1963. The highest annual catch (1954) was associated with an accumulation of trout in the pond (Smith and Saunders, 1967) and heavy effort, the lowest with an exceptionally dry and low-water season in 1961. These data include hatchery-reared trout (Table VII). With the planted trout excluded, the average annual catch was 1528 ± 452.

The 19 years of the study may be divided into three periods: (1) pre-pond, 1947–52; (2) early years after pond formation, 1953–56; and (3) late years after pond formation, 1957–65. Effort and catch during the early years after pond formation were significantly higher ($p < 0.05$) than in the years before or after. The increases mainly resulted from fishing an accumulation of trout in the pond and pool below the dam, 1953–56 (Smith and Saunders, 1967). When effort and catch for the pre-pond and the late post-pond years are compared, the annual average effort was somewhat, but not significantly ($p > 0.05$), greater (966 as against 825 rod-hr) and the average annual catch was somewhat, but not significantly ($p > 0.05$), less (1342 as against 1427 trout) in the post-pond period. Pond formation resulted in only a temporary improvement in catch for the Ellerslie System.

Angling success at Ellerslie may be compared with that at two other artificial Prince Edward Island ponds for which statistics are available. Catch from 23-acre (9.3-ha) Montague Pond varied annually from 3533 to 3819 brook trout over a 3-year period (Smith, 1947). Average rate of capture was 2.3 trout per rod-hour. Creel censuses at Wilmot Pond (13 acres; 5.3 ha), and the upper part of the Wilmot estuary, disclosed a yield to anglers of 8215 brook trout from June 1956 through September 1958 (Smith, 1963). Rate of capture was 1.2 trout per rod-hour. Angling success in these waters and Ellerslie Brook was superior to that in eight natural lakes of Charlotte County, New Brunswick, where rate of capture over a 7-year period (1941–47) was 0.3 per rod-hour, and involved hatchery-reared trout (Smith, 1952). For the most part, the catches from Prince Edward Island were naturally reared fish.

Season of Angling

The brook trout is a cool-water species and, in general, is most successfully fished in spring and early summer. When water temperature exceeds

TABLE VII. Angling statistics for the Ellerslie System. Numbers of planted hatchery-reared trout given in parentheses but included in monthly and seasonal totals.

	No. of trout caught							Fishing effort as rod-days								Fishing effort as rod-hr							
	Apr.	May	June	July	Aug.	Sept.	Season	Apr.	May	June	July	Aug.	Sept.	Season	Per rod-day	Apr.	May	June	July	Aug.	Sept.	Season	Per rod-hr
1947	46	623	181	211	47	27	1,135	99	250	112	80	24	7	572	2.0	113	339	137	134	30	15	768	1.5
1948	37	450	286	163	111	9	1,056	42	154	130	86	39	5	456	2.3	44	252	206	116	83	7	708	1.5
1949	536	842	432	136	43	12	2,001(2)	157	268	170	73	18	6	692	2.9	291	432	281	137	24	11	1,176	1.7
1950	395	753	163	135	77	51	1,574(213)	132	245	76	47	26	12	538	2.9	202	416	113	84	55	19	889	1.8
1951	626	232	177	190	110	111	1,446(61)	193	104	52	50	31	24	454	3.2	308	161	65	82	44	33	693	2.1
1952	353	605	248	269	156	2	1,633(7)	93	139	55	44	35	1	367	4.4	172	228	97	114	104	1	716	2.3
1953	717	560	222	82	233	146	1,960	202	226	132	60	108	52	780	2.5	383	414	159	84	205	76	1,321	1.5
1954	609	832	537	663	120	109	2,870	280	383	216	226	93	39	1,237	2.3	557	699	446	498	158	102	2,460	1.2
1955	450	408	392	162	174	75	1,661(6)	227	307	253	117	101	44	1,049	1.6	389	508	392	184	164	41	1,678	1.0
1956	697	476	384	173	165	132	2,027(141)	307	301	272	174	83	52	1,189	1.7	483	401	368	185	114	83	1.634	1.3
1957	80	978	217	147	142	104	1,668(39)	195	345	128	91	80	15	854	2.0	263	503	208	111	151	38	1,274	1.3
1958	147	260	236	226	124	232	1,225(8)	177	198	121	115	53	63	727	1.7	293	273	124	174	70	109	1,043	1.2
1959	615	134	196	103	110	52	1,210	205	131	152	77	75	53	693	1.7	364	160	160	105	94	55	938	1.3
1960	662	270	253	128	62	54	1,429	296	117	91	91	46	19	660	2.2	510	176	127	101	46	26	986	1.5
1961	99	240	106	196	28	62	731	57	126	66	74	15	24	362	2.0	59	196	83	145	28	34	545	1.3
1962	368	503	475	450	76	218	2,090(555)	103	150	140	166	52	50	661	3.2	144	257	230	245	71	86	1,033	2.0
1963	510	732	376	129	75	208	2,030(541)	108	251	132	84	29	53	657	3.1	198	401	197	149	52	89	1,086	1.9
1964	367	435	401	188	104	84	1,579(119)	79	194	141	78	41	27	560	2.8	132	295	208	133	56	60	884	1.8
1965	311	581	543	143	61	50	1,689(153)	92	199	151	68	33	26	569	3.0	146	370	230	94	32	35	907	1.9
1947–65	7,625	9,914	5,825	3,894	2,018	1,7383	1,014(1,845)	3,044	4,088	2,590	1,801	982	572	13,077	2.4	5,051	6,481	3,831	2,875	1,581	920	20,739	1.5
Per rod-day								2.5	2.4	2.2	2.2	2.1	3.0	2.4									
Per rod-hr																1.5	1.5	1.5	1.4	1.3	1.9	1.5	

TABLE VIII. Angling statistics for stream area flooded by pond and for pond and pool below dam. Native trout only.

	Effort rod-hr	No. of trout caught	Catch per rod-hr	% of total Ellerslie catch
	I. Stream area flooded by pond			
1947	371	558	1.5	49
1948	298	441	1.5	42
1949	586	1015	1.7	51
1950	345	415	1.2	30
1951	344	635	1.8	46
1952	303	719	2.3	44
Avg, 1947–52	375	631	1.7	44
	II. Pond and pool below dam			
1953	1234	1815	1.5	93
1954	2095	2271	1.1	79
1955	1389	1086	0.8	66
1956	1356	1386	1.0	73
1957	1142	1310	1.1	80
1958	753	530	0.7	44
1959	746	950	1.3	79
1960	742	865	1.2	61
1961	399	491	1.2	67
1962	726	802	1.1	52
1963	662	924	1.4	62
1964	598	818	1.4	56
1965	618	862	1.1	63
Avg, 1953–65	958	1085	1.1	74
Avg, 1958–65	656	780	1.2	59

20 C (68 F), brook trout tend to move into cooler waters if possible. Otherwise, as the water temperature rises above 20 C, yet remains below lethal levels, feeding activities of the trout lessen or stop (Baldwin, 1957; Hoar, 1942; Smith, 1960). Angling for brook trout may remain good during the summer where waters are relatively cool, as in many Prince Edward Island streams and ponds.

Early-season angling is an important factor in reduction of available fish. Further, seasonal movements of trout within and between fresh- and salt water, prominent in spring and early summer but minimal in late summer and early autumn (White, 1941; Smith and Saunders, 1958) differentially affect seasonal angling success in different parts of a stream system such as Ellerslie.

Table IX shows that 74% of the effort and 76% of the catch from the Ellerslie System were made during the first half of the angling season (April–June). Much of the effort and catch in the estuary, stream area flooded by the pond, and pool below the dam, was in spring and early summer. These

TABLE IX. Effort and catch at Ellerslie in spring and early summer (April–June) before and after pond formation, 1947–65.

	Entire angling season		April–June			
	Rod-hr	No. caught	Rod-hr	% of total	No. caught	% of total
I. Prior to pond formation (1947–52)						
Estuary	724	1,317	681	94	1,258	96
Stream area flooded	2,247	3,925	1,929	86	3,391	86
Stream area above pond	1,979	3,603	1,247	63	2,336	65
All areas	4,950	8,845	3,857	78	6,985	79
II. After pond formation (1953–65)						
Estuary	1,240	1,724	1,016	82	1,437	83
Pool below dam	3,527	5,111	3,117	88	4,743	93
Pond	8,903	10,357	6,341	71	7,755	75
Stream above pond	2,119	4,977	1,032	49	2,444	49
All areas	15,789	22,169	11,506	73	16,379	74
III. Before and after pond formation						
Entire stream system	20,739	31,014	15,363	74	23,364	75

were areas readily accessible and heavily fished, and in which many of the trout were transients. They also had the highest summer water temperatures. The more spacious pond, with bottom water seldom exceeding 18 C (64 F) (Smith and Saunders, 1967), continued to provide good yields of trout to the anglers well into the summer although there was little recruitment from the stream above at this season (Smith and Saunders, 1967). There was a more sustained effort and catch in the stream above the pond in late summer and early autumn than in the other parts of the system (Table VI). Less accessibility, relatively cool water, more recruitment by growth to the stock of trout of acceptable angling size during the fishing season, and the more resident character of the population contributed to the observed angling pattern in the stream above the pond.

TIME OF ANGLING

Figure 2 illustrates the frequencies of rods fishing during the hours of the day on the Ellerslie System. About 80% of the rods fished during the afternoon and evening.

Much of the fishing effort was applied to the pond, particularly during its early years (Tables II and VII). However, the hourly pattern of angling remained much the same for the system as it had been before the pond. Thus, 45% of the rod-hours was applied to the system between 1 and 6 PM during the pre-pond years (1947–52) and 48% during the early pond years (1953–56).

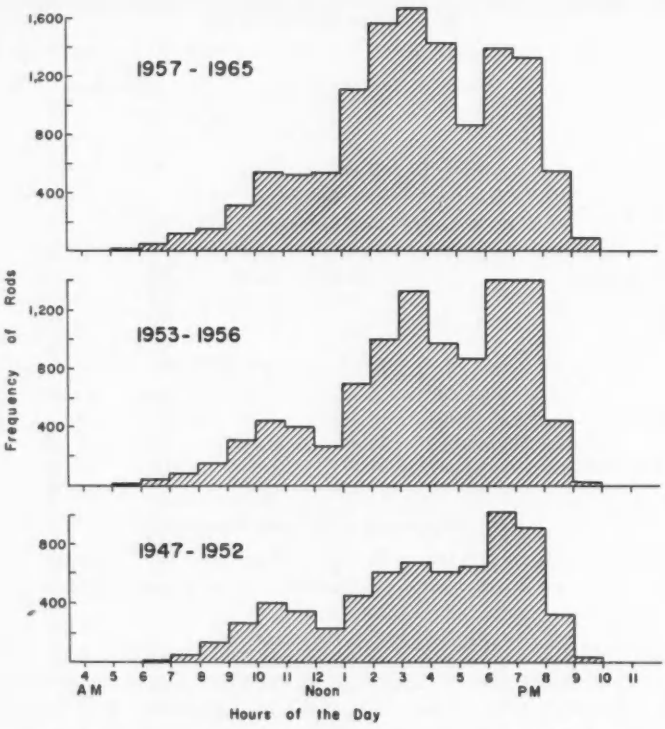

FIG. 2. Frequency of rods fishing during the hours of the day.

The percentage of rod-hours after 6 PM was 34 during the two periods. During the later pond period (1957–65) the effort was somewhat higher in the afternoon (Fig. 2). Even though the pond materially altered the fishing area of Ellerslie, there was little change in the habits of the anglers with respect to the time of day that they fished.

TIME SPENT IN ANGLING

The average time spent by anglers per fishing trip, i.e., per rod-day, is given in Table X by area and month. For the entire study period, the average rod-day was 1.6 ± 0.20 (standard deviation) hr. Of the 13,077 rod-days, only 7% were over 3 hr, 2% over 4 hr, and 1% over 5 hr. Anglers spent somewhat more time per fishing trip on the pond and stream above the pond than elsewhere. For all areas there was little difference in the length of the rod-day from month to month. The average rod-day before and after pond formation was the same (1.6 hr).

TABLE X. Average length of rod-day according to area and season.

I. According to fishing area

	Estuary	Pool below dam	Stream area flooded by pond	Pond	Stream above pond	Ellerslie System
Rod-hr	1,964	3,527	2,247	8,903	4,098	20,739
Rods	1,803	2,648	1,688	4,763	2,175	13,077
Hr per rod	1.1	1.3	1.3	1.9	1.9	1.6

II. According to months in angling season

	Apr.	May	June	July	Aug.	Sept.	All months
Rod-hr	5,051	6,481	3,831	2,875	1,581	920	20,739
Rods	3,044	4,088	2,590	1,801	982	572	13,077
Hr per rod	1.7	1.6	1.5	1.6	1.6	1.6	1.6

ANGLING SUCCESS

Angling success may be gauged by the number of fish caught per rod-day or per rod-hour.

Number caught per rod-day.— The fishing effort as rod-days and the average catch per rod-day are given in Table VII for the month and year. The average catch per rod-day varied between years from 1.6 to 4.4 trout, with an average for all years of 2.4 ± 0.7 (standard deviation). The proportion of successful rods, i.e., rods taking one or more trout per rod-day, is shown in Table XI. It is seen that 65% of the rods caught all of the trout. The successful rods caught trout at the average rate of 3.5 ± 0.6 (standard deviation) per rod-day.

The proportion of successful rods was significantly ($p < 0.05$) lower for 8 years after pond formation (1953–60). Factors, such as population size, modes of angling, and others, determine whether an angler takes trout. At Ellerslie, differences between pond and stream fishing are advanced as important factors resulting in the lower proportion of successful anglers after pond formation. Whereas the angler on the pond usually sat in an anchored boat or at a favoured spot on the shore of the pond, and waited for the trout to come to his lure, the angler on the stream moved from one likely fishing area to another, and probably presented the lure to a greater number of fish. In later years (1962–65) when fishing effort on the pond had declined and that on the stream had again increased, the proportion of successful anglers on the system increased to above pre-pond levels (Tables II, VI, and XI). A larger population of trout in the part of the stream affected by stream improvement probably contributed (Smith and Saunders, 1967).

TABLE XI. Number of rods taking one or more trout (successful rods) and average rate of capture per rod-day.

	Total no. of rods	Rods taking one or more trout (successful rods)	% of successful rods	Catch per rod-day for all rods	Catch per rod-day for successful rods
1947	572	312	55	2.0	3.6
1948	456	305	67	2.3	3.5
1949	692	533	77	2.9	3.8
1950	538	379	70	2.9	4.2
1951	454	324	71	3.2	4.5
1952	367	305	83	4.4	5.3
1953	780	500	64	2.5	3.9
1954	1,237	793	64	2.3	3.6
1955	1,049	542	52	1.6	3.1
1956	1,189	540	45	1.7	3.8
1957	854	451	53	2.0	3.7
1958	727	371	51	1.7	3.3
1959	693	392	57	1.7	3.1
1960	660	391	59	2.2	3.7
1961	362	260	72	2.0	2.8
1962	661	567	86	3.2	3.7
1963	657	574	87	3.1	3.5
1964	560	444	79	2.8	3.6
1965	569	477	84	3.0	3.5
1947–65	13,077	8,460	65	2.4	3.5

Number caught per rod-hour.— The numbers of native and hatchery-reared trout taken per rod-hour are given in Tables II–VII.

The average annual catches of native trout per rod-hour from the several fishing areas are brought together in Table XII. Before the pond was formed, 1947–52, the average annual catches per rod-hour in the estuary, in the stream area later flooded by the pond, and in the stream later above the pond, were not significantly different ($p' > 0.05$). After the pond was formed, the catches per rod-hour on the stream above the pond improved significantly ($p < 0.05$). In the estuary, however, the catches per rod-hour of native trout significantly declined ($p < 0.01$) with pond formation, particularly in the late years. After the pond was formed, the average catches per rod-hour on the stream above the pond were significantly greater ($p < 0.01$) than for the pond or pool below the dam. For the entire system, the average annual catch per rod-hour of native trout was less ($p < 0.01$) after pond formation.

Allen and Cunningham (1957) thoroughly discuss rates of capture as indices of population size and of angling success. Capture per rod-hour is most meaningful as an index to compare angling success between years and fishing areas when such factors as modes of fishing, periods of the day in fishing, and

TABLE XII. Average annual catch per rod-hour of native Ellerslie trout.

	Pond	Pool below dam	Stream area later flooded	Estuary	Stream above pond	Entire system
1947			1.5	2.3	1.4	1.5
1948			1.5	1.6	1.5	1.5
1949			1.7	1.4	1.8	1.7
1950			1.2	2.0	1.6	1.5
1951			1.8	1.7	2.3	2.0
1952			2.4	2.3	2.2	2.3
1953	1.5			1.5	2.0	1.5
1954	1.1			1.3	2.4	1.2
1955	0.8			1.3	2.7	1.0
1956	0.9	1.2		1.4	2.5	1.2
1957	1.0	1.3		1.1	3.3	1.3
1958	0.8	0.6		1.3	3.3	1.2
1959	1.5	1.6		1.2	1.5	1.3
1960	1.0	1.1		1.6	2.2	1.5
1961	1.3	0.8		1.4	1.7	1.3
1962	1.5	0.9		0.8	2.5	1.5
1963	1.9	1.3		0.2	2.0	1.4
1964	1.4	1.2		0.8	2.5	1.7
1965	1.4	1.7		0.5	2.1	1.5

length of fishing day are comparable. They found for angling in New Zealand waters that rate of capture decreased with increasing length of rod-day.

In Figure 3 the average catch per rod-day at Ellerslie has been plotted against length of rod-day. Proportionality between catch and length of rod-day was good up to a rod-day length of about 3 hr, reasonably good to 5 hr, then largely lost with longer rod-days. "If the graph of catch against length of day can be represented by a straight line..., the curve for catch per rod-hour against length of day will fit a rectangular hyperbola, with rate of catch declining rapidly at first, and then more slowly, from an initial theoretical value of infinity for days of zero length and finally becoming asymptotic to a horizontal line at a level corresponding to the slope of the catch-day line." (Allen and Cunningham, 1957, p. 23.) Figure 3 shows that for length of rod-days between 1 and 3 hr, the catch per rod-hour remained quite constant at about 1.5.

The angling statistics for rod-days of 1–3-hr duration, which accounted for a large proportion of the effort and catch and for which catch per rod-hour was relatively constant, are given in Table XIII. Catch per rod-hour for rod-days of 1–3 hr and for all rod-days are also compared in Table XIII. For 10 of the 19 years of observations there was little difference in the average annual catch per rod-hour. The maximal difference was 0.3 in 1956. As already noted, only 7% of the rod-days were over 3-hr duration, and 1% over 5 hr.

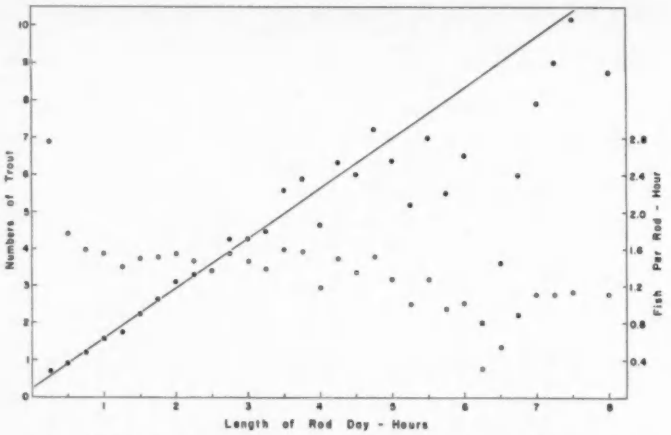

FIG. 3. Catch of trout per rod-day and per rod-hour plotted against length of rod-days at quarter-hour intervals. Open circles — catch per rod-day; solid circles — catch per rod-hour.

Although there was increasing lack of proportionality between catch and length of rod-day with the longer rod-days, and a resulting decline in catch per rod-hour, the effort and catch for these longer rod-days were not sufficient when compared with the whole to affect appreciably the average annual catch per rod-hour when based on all rod-days. This situation, and little change in the time and mode of fishing (Fig. 2) over the years, permitted the use of the catch per rod-hour, based on all rod-days, as an index of the comparative angling success between years and fishing areas.

EFFECTIVENESS OF ANGLERS IN CROPPING ELLERSLIE TROUT

Ellerslie System.— From 1953 through 1964, anglers at Ellerslie recaptured 8560 of 23,040 trout that had been captured by electrofishing or in traps, tagged, and released (Table XIV). The overall average annual exploitation rate was 37%, varying from 15 to 50%.

The rates of exploitation by anglers in Table XIV are the annual rates. A large proportion of the trout were tagged from late summer to early winter. Some of these tagged trout doubtless died over the winter before the following angling seasons. Accordingly, the rates of exploitation were actually higher than those given in Table XIV.

Pond.— Table XV provides data on the rates of exploitation in the pond of tagged trout known to have entered the pond. The annual rates of exploitation averaged 40%, with variation from 18 to 53%. The annual rates were affected, among other factors, by differences in natural mortalities and in the numbers of trout that ran from the pond. The percentage of tagged trout that left the pond in 1954 and 1955 was 22 in both years, whereas that in 1956 was 37 (Smith and Saunders, 1967).

TABLE XIII. Angling statistics for rod-days of 1–3-hr duration.

	Rod-days		Rod-hr		Catch		Catch per rod-hr	
	1–3-hr duration		1–3-hr duration		1–3-hr duration		1–3-hr duration	
	Of 1–3-hr duration	% of total rod-days	For rod-days of 1–3-hr duration	% of total rod-hr	For rod-days of 1–3-hr duration	% of total catch	For rod-days of 1–3-hr duration	For all rod-days
1947	453	76	658	86	995	88	1.5	1.5
1948	329	72	544	77	817	77	1.5	1.5
1949	538	78	899	76	1,509	75	1.7	1.7
1950	388	72	648	73	1,192	76	1.8	1.8
1951	315	69	499	72	1,040	72	2.1	2.1
1952	246	70	436	61	1,095	67	2.5	2.3
1953	546	70	951	72	1,363	70	1.4	1.5
1954	847	68	1,337	54	1,782	62	1.3	1.2
1955	782	75	1,350	80	1,276	77	0.9	1.0
1956	765	64	1,244	76	1,442	71	1.6	1.3
1957	551	65	934	73	1,225	73	1.3	1.3
1958	484	67	819	79	1,027	84	1.3	1.2
1959	445	64	704	75	915	76	1.3	1.3
1960	443	67	732	74	1,148	80	1.6	1.5
1961	258	71	390	72	512	70	1.3	1.3
1962	495	75	846	82	1,674	80	2.0	2.0
1963	465	71	823	76	1,491	73	1.8	1.9
1964	397	71	679	77	1,085	69	1.6	1.8
1965	402	71	697	77	1,312	78	1.9	1.9
1947–65	9,131	70±3.8[a]	15,190	74±6.8	22,900	75±5.9	1.5	1.5

[a]Standard deviation.

TABLE XIV. Exploitation of tagged trout by anglers in the Ellerslie System.

Year of tagging	No. tagged (native trout)	No. subsequently caught in Ellerslie System	
		No.	%
1953	3,199	1,365	43
1954	2,710	1,348	50
1955	2,623	1,071	41
1956	2,992	1,245	42
1957	1,506	450	30
1958	1,538	564	37
1959	1,435	470	33
1960	1,600	234	15
1961	1,327	432	33
1962	1,607	698	43
1963	1,086	311	29
1964	1,417	372	26
1953–64	23,040	8,560	37

The pond was drained on September 15, 1958, September 13, 1962, and September 30, 1963. Screens at the dam prevented escapement of trout as the pond was being drained. Trout left in the stream bed were captured by electrofishing, which has been shown to be an effective method of capture in Ellerslie waters (Saunders and Smith, 1954). The numbers of trout at the

TABLE XV. Exploitation by anglers of tagged trout in the pond.

	No. of tagged trout into pond	Angled from pond	
		No.	%
1952	1,051	533	51
1953	1,798	654	36
1954	2,027	1,089	53
1955	1,365	631	46
1956	1,921	345	18
1957	398	141	35
1958	263	114	43
1959	167	64	38
1960	174	60	35
1961	194	69	36
1962	484	253	52
1963	169	89	53
1952–63	10,011	4,042	40

three drainings were 73, 19, and 29 for the years 1958, 1962, and 1963. Rates of exploitation during these three seasons were 43, 52, and 53% (Table XV). Angling was a prominent factor in reducing the pond population each year. In spite of the near depletion, anglers still made good catches late in the season, pointing to the high vulnerability of trout to anglers in the pond at the moderate water temperatures of early autumn.

Stream above pond.— Table XVI shows the exploitation of trout tagged annually in the stream above the pond. They were tagged as yearlings (age I)

TABLE XVI. Exploitation by anglers of trout tagged in stream above pond.

Year of tagging	No. tagged	No. subsequently angled in Ellerslie System	% of tagged trout angled
1953	1,369	451	33
1954	831	200	24
1955	1,428	383	27
1956	1,225	348	28
1957	907	176	19
1958	1,034	271	26
1959	991	213	21
1960	1,424	148	10
1961	1,087	305	28
1962	1,144	393	34
1963	941	226	23
1964	1,341	353	26
1953–64	13,722	3,467	25

or older, near or after the close of the angling seasons. Almost all of the recaptures by anglers were during the year after tagging, with only a few in subsequent years. The average exploitation rate for the trout tagged in the stream was 25%, varying from 10 to 34%. Of the 3467 recaptures by anglers (1953–64), 1810 (52%) were made in the stream; the others were made after the trout had moved downstream into the pond, the pool below the dam, or the estuary.

Exploitation rates given in Tables XIV, XV, and XVI are for tagged trout. To what extent tagging may have affected their catchability was not well established. Approximately equal numbers of tagged and untagged trout among those left in the pond when drained at the close of angling seasons suggests that they were about equally catchable.

Exploitation rates of tagged and untagged trout in the stream above the pond were also derived. The numbers of trout in 14 blocked-off, 50-yard sections of the stream were annually assessed by electrofishing in late summer and early autumn (Saunders and Smith, 1954). These sections were representative of the stream length (6300 yards) that supported yearling and older

trout. To estimate the numbers of yearling and older trout present in the stream at the beginning of angling seasons, the numbers of trout in the estimates were added to those that were angled from the stream each year, then subtracting those known to have moved downstream early in the angling seasons (April) before being subjected to much angling pressure. The estimates did not account for natural mortalities in the stream during the angling seasons. When only trout of 14 cm or over in fork length, which were those usually taken by anglers, were considered, the exploitation rates averaged 25% (11–34%) (Table XVII).

TABLE XVII. Exploitation by anglers of tagged and untagged trout 14 cm and over in fork length in stream above pond.

	Estimated no. 14 cm and over in stream at start of angling season	Recorded no. 14 cm and over angled from stream	% angling mortality
1953	680	82	12
1954	1,050	305	29
1955	1,460	323	22
1956	1,440	231	16
1957	930	203	22
1958	1,580	377	24
1959	1,080	103	10
1960	1,700	365	21
1961	790	104	13
1962	1,420	393	28
1963	1,210	527	44
1964	1,230	368	30
1953–64	14,570	3,381	23

SIZE OF ANGLED TROUT

The average fork lengths of trout according to fishing areas and years are given in Table XVIII. The averages are based on 2978 trout from the estuary, 5304 from the pool below the dam, 3834 from the stream area later flooded by the pond, 9708 from the pond, and 7161 from the stream above the pond.

The overall average fork length of angled native trout was 17.2 cm. (For Ellerslie trout of this size, total length equals 1.03 × fork length.) Smith and Saunders (1958) found that the average fork length of angled native trout from Ellerslie was 15.7 cm for age I, 17.1 cm for age II, and 21.6 cm for age III. Accordingly, the angled trout were dominantly age II (Fig. 4). However, beginning noticeably about 3 years after pond formation (1956), there was an increasing proportion of age I (yearling) trout, particularly in the catches from the stream above the pond (Table XVIII; Fig. 4).

TABLE XVIII. Average fork length in centimetres of angled native brook trout from the Ellerslie System (2.54 cm = 1 inch). Averages including planted hatchery-reared trout given in parentheses.

	Estuary	Pool below dam	Stream area flooded	Pond	Stream above pond	All areas
1947	15.7	–	17.2	–	18.5	17.8
1948	16.9	–	17.3	–	17.6	17.3
1949	16.9	–	17.4	–	17.7	17.4
1950	16.7	–	16.3(16.2)	–	16.8(16.7)	16.6(16.5)
1951	17.6	–	17.3	–	17.8	17.2
1952	19.1	–	18.7	–	18.7	18.7
1953	18.8	–	–	17.5	17.5	17.5
1954	17.3	–	–	17.9	17.1	17.8
1955	19.9	–	–	17.9	16.7	17.8
1956	18.7	18.1	–	15.4(15.3)	14.4	16.8
1957	17.5	16.3	–	16.3	14.6	16.1
1958	18.5	17.2	–	17.1	15.3	16.5
1959	18.2	16.9	–	17.6	14.9	16.9
1960	18.7	18.2	–	17.0	15.8	17.2
1961	19.1	17.1	–	15.6	14.5	16.0
1962	16.7(16.4)	17.2(15.2)	–	15.9	15.8	16.2(15.6)
1963	18.5(22.0)	17.7(20.1)	–	17.6	15.8	17.1(18.6)
1964	20.1(19.2)	18.1(17.3)	–	17.2	13.8	16.2
1965	19.0(17.3)	16.4(16.0)	–	15.3	14.6	15.6(15.5)

The average length of the native trout angled from the stream above the pond was significantly ($p < 0.01$) shorter after pond formation; 17.9 cm before (1947–52) and 15.5 cm after (1953–65). The average length of trout angled from the pond did not change significantly ($p > 0.05$). Native trout taken by anglers from the estuary were significantly ($p < 0.05$) longer in the post-pond period. However, significantly fewer native trout were caught

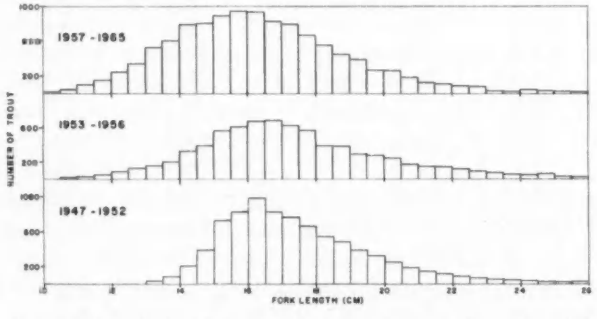

FIG. 4. Length (fork) frequencies in centimetres of trout angled from the Ellerslie System.

in the estuary after the pond was formed. Trout from the estuary were 15% of the total catch in pre-pond years. Their contribution dropped to 6% after the pond (1953–60) and further to 3% during the last years of the study (1961–65). The average length for all trout caught from the Ellerslie System significantly ($p < 0.05$) declined during the years after pond formation, from an average of 17.6 cm in the pre-pond years to 16.8 cm in the post-pond period (Table XVIII). This decrease is largely attributed to a reduction in the size of fish captured from the stream above the pond with advance of years from pond formation.

Angling Catches by Weight

Table XIX gives the weight of trout taken annually from the several fishing areas. The annual yield by weight for the system was a maximum in 1954, the 2nd year after pond formation. However, the second and third best yields were in pre-pond years, 1949 and 1952. In the later pond years, 1957–65, the annual yields by weight declined significantly ($p < 0.01$). These lower

Table XIX. Weight of native brook trout angled from the Ellerslie System, by year and fishing area. Weight in kilograms (0.45 kg = 1 lb).

	Estuary	Pool below dam	Stream area flooded	Pond	Stream above pond	Entire system
1947	3.1	–	36.5	–	43.7	83.3
1948	12.2	–	28.5	–	27.7	68.4
1949	18.0	–	67.5	–	48.3	133.8
1950	24.8	–	28.6	–	32.8	86.2
1951	12.2	–	43.8	–	44.6	100.6
1952	15.5	–	56.8	–	58.4	130.7
1953	3.9	–	–	105.2	5.2	114.3
1954	15.1	–	–	145.2	17.3	177.6
1955	16.2	–	–	73.6	19.2	109.0
1956	18.0	46.9	–	29.7	9.0	103.6
1957	3.6	33.8	–	30.9	8.6	76.9
1958	12.1	12.3	–	19.6	22.8	66.8
1959	6.8	15.4	–	35.4	5.3	62.9
1960	9.2	28.5	–	21.5	18.5	77.7
1961	5.4	9.1	–	14.9	6.0	35.4
1962	0.9[a]	11.4[b]	–	24.0	31.4	67.7
1963	4.4[a]	14.3[b]	–	43.7	23.1	85.5
1964	3.3[a]	19.7[b]	–	25.9	18.8	67.7
1965	3.0[a]	25.8[b]	–	16.1	26.8	71.7
Totals	187.7	217.2	261.7	585.7	467.5	1719.8

[a]Trout planted in estuary yielded in addition 2.5 kg in 1962, 21.4 kg in 1963, 0.7 kg in 1964, and 1.7 kg in 1965.

[b]Trout planted in estuary yielded in addition 20.6 kg in 1962, 17.4 kg in 1963, 6.4 kg in 1964, and 6.0 kg in 1965.

yields by weight resulted from the shorter average length of the trout taken from the system in the latter period. Accordingly, the pond did not improve the harvest by weight of native trout from the system, even though the annual effort and catches by number were not significantly lower in the later years after pond formation.

The 6-inch minimum size limit (total length) on trout was removed in 1955. Doubtless this was a factor in the shorter average length of trout angled in later pond years, aside from any direct or indirect effect the pond may have had upon average size and capture of trout. However, within the years that no size limit was in effect, the presence of the pond did not improve the yield by weight of native trout to anglers (Table XIX), nor significantly change effort in angling or catch by number.

CONTRIBUTIONS OF PLANTED HATCHERY-REARED TROUT TO ANGLERS' CATCHES

Hatchery-reared trout were planted in autumn and early winter as follows: (1) in the stream, 1948 — 3320, age 0 (underyearlings); 1949 — 1679, age I (yearlings); 1954 — 4020, age 0; 1963 — 2000, age 0; 1964 — 2000, age 0; (2) in the estuary, 1961 — 1978, age 0; 1962 — 1973, age I; 1963 — 2000, age 0; 1964 — 2000, age 0. The trout were fin-clipped (age 0) or tagged (age I) before being planted.

Plantings in the stream in 1948, 1949, and 1954 consisted of hatchery-reared progeny of native sea-run and non-sea-run trout from Prince Edward Island water (Smith and Saunders, 1958). The purpose was to learn whether sea-running was heritable. Results were inconclusive. Plantings in the estuary were to provide trout for angling there after pond formation almost eliminated the sea-running of native trout from Ellerslie Brook (Saunders and Smith, 1964). In 1963 and 1964 the same numbers of underyearling trout were planted in the estuary and in the stream above the pond.

The numbers of planted trout taken by anglers in the years that these fish were available are shown in parentheses in Tables II–VI. The contributions that the planted trout made to the catches are further shown in Table XX.

The survival to anglers' catches of the yearlings planted in the stream in 1949 was 16% (Saunders and Smith, 1964). These fish largely made up the 14% contribution by planted trout to the total catch in 1950. The best contribution by underyearlings planted in the stream to the total catch was 7% in 1957 (planted in 1954).

An attempt to provide angling in the estuary by planting hatchery-reared stock met with a good measure of success. Underyearlings and yearlings planted in the estuary contributed 7–27% of the total catch from the Ellerslie System from 1962 through 1965 (Table XX). Over 90% of the hatchery-reared trout taken from the estuary in these years had been planted in the estuary.

TABLE XX. Contributions of hatchery-reared trout to the anglers' catches from the Ellerslie System.

	No. of hatchery-reared trout caught	Total no. of trout caught from the system	% contribution of hatchery-reared trout to total catch
1949	2	2001	<1
1950	213	1574	14
1951	61	1446	4
1952	7	1633	<1
1955	6	1661	<1
1956	141	2027	7
1957	29	1668	2
1958	8	1225	1
1962	555	2090	27
1963	541	2030	21
1964	119	1579	7
1965	310	1689	18

DISCUSSION

POND AND POOL BELOW DAM AS FISHING AREAS

Mill ponds on Prince Edward Island streams have long been appreciated for good bfook trout fishing. However, most small water-powered mills were abandoned early in this century. A few mill ponds were maintained for fishing but most were drained. In recent years, with increasing demand for angling, fishing ponds have been formed at old pond sites, or other suitable locations in streams, under private and government auspices. Judged from creel censuses and general reports from anglers, such ponds quite consistently provide good trout fishing (Loftus, MS, 1948; Smith, 1947, 1963). Ellerslie Pond was no exception. Although the relatively high yields during the first 3 or 4 years were not later realized, the pond, and the pool below the dam continued to provide good angling 13 years after they were formed. Accordingly, pond formation on a coastal stream, such as Ellerslie, to provide accessible areas for good trout fishing appears to have merit. However, when the entire stream system is viewed, there are serious reservations.

ANGLING FOR SEA-RUN BROOK TROUT

Some of the brook trout in coastal streams of the Atlantic provinces of Canada run to salt water. Many anglers consider the sea-run trout better to catch and eat. Also, the largest brook trout taken from stream systems are often sea-run individuals. At Ellerslie Brook, the average fork length of the sea-run trout as they entered the brook in pre-pond years was 19.2 cm, with 10% exceeding 25 cm (Smith and Saunders, 1958).

Seaward movements of trout were delayed in the pond and pool below the dam at Ellerslie where heavy mortalities resulted (Smith and Saunders,

1967). In the late years after pond formation, relatively few trout ran to salt water. Accordingly, pond formation almost eliminated angling for native sea-run trout. Maintenance of angling for sea-run trout as a management objective is not compatible with pond formation.

For the most part, trout angled from the pool below the dam were not classed as sea-run. Trout in the pool were primarily those that came over the dam from the pond, and secondarily, those that had gone into the estuary and returned to the pool. The latter fish, properly classed as sea-run trout, became scarce after pond formation (Smith and Saunders, 1967). Anglers annually cropped the trout in the pool effectively, and few of those that came over the dam went farther, into the estuary, or were in the pool sufficiently long to take on characteristics of sea-run individuals.

Yields of Trout to Anglers

With pond formation there was an immediate significant increase in fishing effort and yield of native trout by number and weight. The peak in fishing effort and catch was in the 2nd year after the pond was created. Thereafter, effort and catch declined. Although the average annual effort and catch by number in the later pond years was not significantly different from those in the pre-pond period, the trout caught were significantly shorter, and the annual catches by weight significantly less. Accordingly, pond formation did not result in a sustained improved yield of trout to anglers from the Ellerslie System.

SUMMARY

1. A 5-acre pond formed on the lower part of Ellerslie Brook, Prince Edward Island, temporarily increased the yields of native brook trout, by numbers and weight, to anglers from the stream system — estuary, pond, and stream. The relatively high yields came largely from the pond and a pool that formed below the dam. The peak catch from the pond was during the 2nd year after it was formed.

2. Annual fishing effort, as rod-hours, and yield to anglers, as number of brook trout, were not significantly different in the later years after pond formation from those in the pre-pond period, but the average length of trout was significantly less (partly attributable to the removal of a size limit 3 years after pond formation). The yield to anglers by weight was significantly lower.

3. Pond formation resulted in near loss of angling for the favoured sea-running brook trout.

4. Hatchery-reared brook trout planted directly into the estuary to replace the sea-running individuals made substantial contributions to the anglers' catches from the Ellerslie System. Such trout planted in the stream made minor contributions to the catches.

5. Stream improvement to provide pools and cover for trout in the stream above the pond was followed by an improved catch from that area.

6. The pond created a readily accessible fishing area which continued to provide good catches of native trout by number to anglers, but it is to be emphasized that pond formation did not result, except temporarily, in an improved yield of trout to anglers, either by number or weight from the Ellerslie System — estuary, pond, and stream.

ACKNOWLEDGMENTS

The valuable assistance of Messrs C. R. Hayes and Cyril Williams in the field and Miss Mary Holmes in the laboratory throughout this study is

gratefully acknowledged. Messrs Harold Ross, Fenton Boylan, and Earl Coughlin served as creel-census takers. Their services were provided by the Resource Development Service of the Canada Department of Fisheries. Dr E. L. Cox and Professor H. V. Pritchard were also associated with the field studies in the early years of the study. Colleagues at the Fisheries Research Board of Canada, Biological Station, St. Andrews, N.B., in particular Mr K. Radway Allen, made critical and helpful comments on the preparation of this paper. The figures were prepared by Mr F. B. Cunningham.

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Nucleotide Degradation and Organoleptic Quality in Fresh and Thawed Mackerel Muscle Held at and above Ice Temperature[1]

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ABSTRACT

In mackerel, by the time of initial sampling, adenine nucleotides had been deaminated to inosine monophosphate (IMP) in the ordinary muscle; in the red muscle the degradative sequence was even more advanced, as indicated by high initial levels of inosine. Postmortem rates of degradation of IMP to hypoxanthine through inosine were similar in both types of muscle; at ice temperature the rates were slower than in cod but faster than in swordfish. A delay in icing of 6–8 hr after catching accelerated the gradual decline in eating quality with replacement of the characteristic fresh mackerel flavor by tastelessness. IMP dephosphorylation paralleled development of tastelessness, and spoilage (organoleptic) had developed prior to accumulation of appreciable amounts of hypoxanthine. At higher temperatures, 5–20 C, rates of IMP dephosphorylation, hypoxanthine accumulation, and quality loss were markedly increased. Thawing did not influence subsequent deterioration rates, but ascorbic acid dips delayed darkening of the flesh in the thawed samples.

Excellent correlation of taste with both IMP and hypoxanthine content, and with various simple measures of IMP dephosphorylation, was obtained under the various handling conditions investigated, including delayed icing, holding at elevated temperatures, and after thawing. The simple tests — ultraviolet absorption at 248 mμ of a Dowex treated perchloric acid extract, and ratio of ultraviolet absorption of extracts at 251 mμ after Dowex treatment to that before treatment — proved as good indices of progressive quality loss to the unacceptability level as the more complex estimation of IMP or hypoxanthine.

INTRODUCTION

Considerable enzymic dephosphorylation of inosine monophosphate (IMP) via inosine (Ino) to hypoxanthine (Hx) occurs within the period of edibility during iced storage and these changes are implicated in the loss of fresh flavors and development of undesirable ones in several fish species (Jones et al., 1964; Spinelli et al., 1964; Jones, 1965a; Spinelli, 1965; Dyer et al., 1966). This work supported the use of Hx as an index of iced storage time and possibly of quality. A simple estimate of Ino plus Hx — the measurement of ultraviolet absorption at 248 mμ of a perchloric acid extract shaken with Dowex to remove nucleotides — was also suggested as an index (Jones and Murray, 1964; Dyer et al., 1966); use of the ultraviolet absorption of the combined Ino and Hx fraction after column chromatographic separation has also been proposed (Saito et al., 1959).

Information was lacking on the rate and extent of nucleotide and nucleoside degradation in the Atlantic mackerel (*Scomber scombrus*) and its

[1]Received for publication April 24, 1967.
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J. Fish. Res. Bd. Canada, 25(2): 239–253, 1968.
Printed in Canada.

possible association with undesirable flavors and with quality in this important food fish. Also, nucleotide degradation at temperatures above 0 C appears to have been little studied, nor has the effect on nucleotide catabolism of adverse handling conditions — delay in icing, prolonged exposure to high ambient temperatures, freezing, and thawing — to which fish may be subjected under normal commercial practices of handling and marketing. Consequently, these factors have been investigated in mackerel to obtain a general picture of nucleotide degradation in relation to eating quality. The antioxidant ascorbic acid was tried in some experiments since it might prevent or delay rancidity development which could mask nucleotide-related taste changes. Since the sequence of nucleotide changes occurred much earlier in the red than in the white muscle of swordfish (Dyer et al., 1966) the red muscle was also investigated in mackerel where it comprises 11–27% of the edible weight (Mannan et al., 1961).

EXPERIMENTAL

Unless otherwise indicated, mackerel were obtained from a commercial fisherman at Terence Bay, N.S., during June and July 1966. After transportation in ice to the laboratory, the fish were headed, gutted, washed, and individually packaged in heat-sealed polyethylene bags for storage at ice temperature or above, or packaged in sealed laminated polyethylene (Mylar) pouches for freezing and subsequent storage.

For sampling, portions of the white muscle weighing 3–5 g were cut from the dorsal area near the midsection. To reduce the effect of individual variation in fish, samples from three fish for expt. A and from two fish for expt. B and C were pooled and purine compounds extracted in cold 3% perchloric acid as described previously (Fraser et al., 1965). From these same two or three fish, dark-red lateral line muscle samples from the same area were treated similarly. The remaining flesh was used for taste panel testing; consequently, different fish were used at each sampling period. Analyses for IMP, Ino, and Hx were carried out on the neutralized extracts (Dyer et al., 1966). Homogenates of 2–3 g muscle with 10 volumes of 0.005 N sodium iodoacetate (Marsh, 1952) were used for *p*H determination. Histidine was estimated in the neutralized perchloric acid extracts by the method of Albanese et al. (1945) as modified by Wood (1958). Lactate was determined enzymatically (Hohorst, 1963). Thiobarbituric acid values (TBA) were determined by the distillation procedure of Tarlagdis et al. (1960); propyl gallate and EDTA were added to the muscle samples to inhibit oxidation during the blending and distillation (Castell et al., 1966).

For organoleptic evaluation, the fish from which samples had been pooled for analysis were individually graded for cooked texture, flavor, and overall grade by an eight-member analytical taste panel. Scores may range from 100 to 0, denoting perfect to badly spoiled quality respectively, the limit of acceptability being about 40. The fish, individually wrapped in aluminum foil,

were cooked in boiling water for 12–15 min; uniform-sized pieces of the ordinary muscle were immediately taste-tested. Panel techniques and methods of scoring have been described previously (Dyer et al., 1964). Mackerel both strictly fresh and of poor quality were used in taste panel training.

RESULTS

Effect of Delay in Icing on the Changes during Subsequent Iced Storage (Expt. A)

In commercial practice (eastern Canada), mackerel are landed from the traps without ice and thus may be exposed to high ambient temperatures for several hours before being lightly iced for transportation to markets. To examine the effect of such an initial delay in icing on the nucleotide changes and on quality during subsequent iced storage, one lot of fish was exposed after catching to ambient temperatures ranging from 13 to 20 C for a period of 7 hr before processing and storage in ice, whereas a second lot from the same catch was iced immediately upon boating. Since in actual practice the period at high ambient temperature before marketing may be prolonged, nucleotide catabolism was also followed at 20 C in mackerel from the same lot un-iced at catching.

IMP in the ordinary muscle, initially at a high level, appears to be completely dephosphorylated slightly earlier in the mackerel held at 13–20 C for 7 hr before icing than in the fish iced at catching, although the regression lines do not show significant differences (Fig. 1). In both of these lots Ino reached a maximum of about 4 μmoles/g at 12 days. Hx accumulated at about the same rate in both lots, reaching levels of 2–5 μmoles/g at 20 days. Some scatter of points is evident in the results, probably indicating variation among fish which was not averaged out by the pooling of samples from three fish.

In the red muscle IMP had already been almost completely dephosphorylated at the initial sampling. Ino reached a maximum at 5–7 days, and was degraded to Hx in 20–23 days in both lots. The rate and extent of Hx formation was similar in red and in ordinary muscle.

In marked contrast to both lots of iced mackerel, breakdown of IMP and Ino in the fish held at 20 C was rapid, being complete in 3 days; Hx increased to high levels, 7 μmoles/g or greater, at 5 days in both ordinary and red muscle.

The principal change in eating quality accompanying the nucleotide changes in the iced mackerel was a gradual loss of the fresh characteristic mackerel flavor with development of tastelessness, which reduced taste scores from 95 to 50–60% after 7–9 days (Fig. 4*a*). In the fish iced from time of catching, this neutral condition persisted until 19 days, when they were rejected because of appearance of spoilage odors and flavors. Some bitterness was noted at this stage by some of the tasters. The mackerel in which icing was delayed for 7 hr reached the limit of acceptability in 9–12 days and were lower in taste score from those iced throughout after about 10–12 days (significantly lower, $P = 0.05$, at 7, 12, and 14 days as indicated by 95% con-

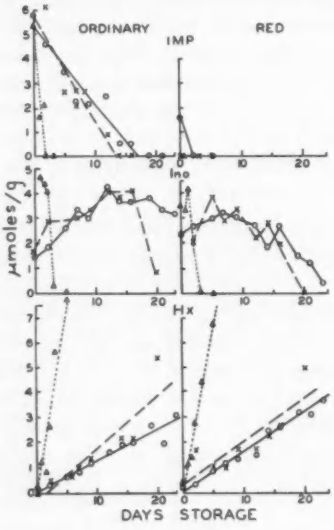

FIG. 1. Nucleotide degradation in ordinary and red muscle of mackerel (expt. A); ○———○ iced immediately after catching; ×— —× held at ambient temperature (13–20 C) for 7 hr, then iced; △.....△ held at ambient temperature (13–20 C) for 7 hr, then held at 20 C. Regression lines for IMP and for Hx are drawn. Statistical data for these lines are tabulated as follows.

	Treatment of fish	Regression equation	$\bar{x}$ (*days*)	$\bar{y}$ (*μmoles/g*)	Goodness of fit[a] (*t*)	95% confidence interval of mean at midpoint	*n*
IMP (linear)							
Ordinary	Iced	Y = 5.25–0.31X	8.2	2.75	7.65***	±0.50	8
	Delayed iced	Y = 5.67–0.41X	7.0	2.76	6.4**	±0.73	7
	20 C	(Y = 5.68–3.3X)	1.0	2.3	3.15 ns	(±2.4)	4
Hx (linear)							
Ordinary	Iced	Y = 0.12X+0.07	11.7	1.49	11.1***	±0.18	11
	Delayed iced	Y = 0.21X–0.40	9.5	1.62	5.14**	±0.61	9
	20 C	Y = 1.72X–0.34	2.3	3.62	3.49*	±1.49	5
Red	Iced	Y = 0.15X+0.09	11.7	1.89	15.3***	±0.17	11
	Delayed iced	Y = 0.17X+0.31	9.5	1.93	4.38**	±0.57	9
	20 C	Y = 1.40X+0.11	2.3	3.33	5.09*	±1.30	5
Hx (log [Hx times 100] vs. time)[b]							
Ordinary	Iced	Y = 0.051X+1.44	11.7	2.04	7.21***	±0.12	11
	Delayed iced	Y = 0.070X+1.35	9.5	2.02	7.62***	±0.13	9
	20 C	(Y = 0.35X+1.40)	2.3	2.21	2.34 ns	(±0.7)	5
Red	Iced	Y = 0.050X+1.57	11.7	2.15	7.80***	±0.11	11
	Delayed iced	Y = 0.041X+1.80	9.5	2.19	4.46**	±0.14	9
	20 C	Y = 0.18X+2.00	2.3	2.43	3.95*	±0.22	5

[a]Goodness of fit and 95% confidence intervals of the mean calculated according to Snedecor (1956), *** = $P<.001$, ** = $P<.01$, * = $P<.05$.

[b]Log Hx vs. log time and the reciprocal of Hx vs. time did not yield as good a fit to the data; see text.

fidence interval of the means). In this and succeeding lots, texture scores declined on storage but did not reach the unacceptability level. Consequently, the decline in overall grade scores closely paralleled that in taste scores for both lots of fish.

Changes during Storage at Temperatures above 0 C (Expt. B)

For this experiment, commercially handled whole mackerel were obtained from a local retail outlet approximately 8 hr after catching; they had been landed without ice, and then lightly iced for transport. After processing at the laboratory, one lot was stored in ice and further lots in constant temperature cabinets at 5, 10, and 15 C.

Nucleotide catabolism rates were markedly greater the higher the temperature (Fig. 2). Peak levels of Ino were reached just prior to the disappearance of IMP. As in the previous experiment the rates of Hx accumulation were not greatly different in red and ordinary muscle, and ultimately reached levels of 5 μmoles/g or greater.

Taste scores decreased progressively to the limit of acceptability at about 10 days in the iced fish, and about 8 days at 5 C (Fig. 4*b*). At 10 C the rating was "fair" at 3 days, and inedible at 6 days with some overtones of bitterness. Unfortunately, absence of personnel made taste panel testing impossible at 4 and 5 days. Since the 15 C samples were unfit for cooking at 6 days because of advanced spoilage, insufficient scores are available to yield significant results of spoilage rate at this temperature.

Muscle *p*H values in these fish were lower initially, *p*H 5.85–6.10, and less variable than those in the fish of the previous experiment (*p*H 6.3–7.0). The *p*H remained below 6.2 during storage at the various temperatures until spoilage was well advanced, when it rose to levels ranging from 6.3 to 7.0. Initial lactate contents, 815 and 380 mg/100 g in the ordinary and red muscle respectively, were high, and in general appeared to decrease slightly with storage time. Such high lactate levels, as well as the high initial IMP content observed, indicated considerable antemortem struggle of the fish in the nets. Histidine levels in ordinary muscle ranged from 400 to 600 mg/100 g (average 499, SD ± 91 for 23 samples), and showed no tendency to change on storage; thus, this possible flavor constituent did not appear to be involved in flavor changes. Initial levels in the red muscle were very much lower, approximately 100, but increased on storage to levels nearly comparable to those in the ordinary muscle, probably as a result of diffusion.

Changes on Storage at 5 C in Frozen Mackerel Thawed at 5 and 18 C (Expt. C).

Commercially caught mackerel, landed without ice and further subjected to ambient temperatures for at least 6 hr, were processed, packaged in laminated polyethylene bags, quick frozen in a plate freezer, and placed in storage at −26 C for 6 weeks. One lot was then thawed slowly in air in a cabinet maintained at 5 C and a second lot thawed quickly in running water at 18 C (24

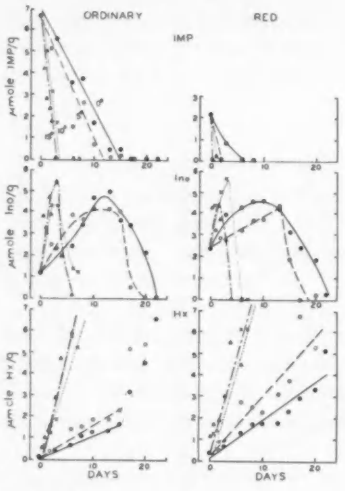

FIG. 2. Nucleotide degradation in ordinary and red muscle of mackerel held at 0–15 C (expt. B); ●——● 0 C; ○– – –○ 5 C; ×.....× 10 C; △–·–·–△ 15 C. Regression lines for IMP and for Hx are drawn. Statistical data for these lines are tabulated as follows.

	Treatment of fish (C)	Regression equation	$\bar{x}$ (*days*)	$\bar{y}$ (*μmoles/g*)	Goodness of fit[a] (*t*)	95% confidence interval of mean at midpoint	*n*
IMP (linear)							
Ordinary	0	Y = 6.80–0.46X	7.0	3.61	13.8***	±0.40	8
	5	Y = 6.44–0.54X	5.71	3.36	7.0***	±0.91	7
	10	Y = 7.28–1.94X	1.36	4.64	11.6**	±0.47	5
	15	Y = 7.15–2.86X	1.06	4.12	4.6**	±1.01	6
Hx (linear)							
Ordinary	0	Y = 0.09+0.104X[b]	6.98	0.80	14.6***	±0.09	8
	5	Y = 0.16+0.15X[b]	6.85	1.17	9.6***	±0.20	8
	10	Y = 0.81X–0.02	2.83	2.26	8.2***	±0.63	7
	15	Y = 1.10X–0.34	2.11	1.99	13.8***	±0.33	9
Red	0	Y = 0.18X+0.11	10.4	1.97	8.6***	±0.34	11
	5	Y = 0.28X+0.20	9.2	2.76	6.8***	±0.63	10
	10	Y = 0.91X–0.34	2.83	2.27	6.5**	±0.91	7
	15	Y = 0.88X+0.36	2.11	2.21	5.3**	±0.68	9
Hx (log [Hx times 100] vs. time)							
Ordinary	0	Y = 1.18+0.077X	10.4	1.98	10.3***	±0.12	11
	5	Y = 1.26+0.083X	9.2	2.02	6.6***	±0.19	10
	10	Y = 1.24+0.246X	2.83	1.94	4.4**	±0.35	7
	15	Y = 1.35+0.30X	2.11	1.98	4.2**	±0.30	9
Red	0	Y = 1.65+0.049X	10.4	2.16	8.3***	±0.10	11
	5	Y = 1.67+0.064X	9.2	2.26	6.2***	±0.16	10
	10	Y = 1.56+0.18X	2.83	2.08	5.4**	±0.22	7
	15	Y = 1.77+0.20X	2.11	2.18	3.8**	±0.21	9

[a]See footnote a, Fig. 1.
[b]To 15 days only.

hr and 70 min respectively for the internal temperature to reach 5 C). The thawed fish were then held at 5 C, a temperature comparable with that in many retail storage cabinets where such thawed fish might be held in practice. Another lot of mackerel from the same catch was given a 30-sec dip in a 0.5% ascorbic acid solution before packaging and freezing; these were thawed slowly in air at 5 C as above.

A small decrease in IMP level and corresponding increase in Ino occurred during the initial freezing and 6 weeks storage (Fig. 3), and during thawing, especially in the samples thawed rapidly at 18 C.

During subsequent storage at 5 C, IMP dephosphorylation was completed earlier in the rapidly thawed samples but no differences were evident with Ino or Hx. Hx accumulated at the same rate in both red and ordinary muscle. Thawing rate did not influence the subsequent eating quality changes in the thawed fish at 5 C (Fig. 4*c*) as taste scores were not significantly different ($P = 0.05$); all samples had become unacceptable by 8 days.

Treatment with ascorbic acid did not significantly influence nucleotide degradation or taste scores. It did, however, prevent the marked darkening of the ordinary muscle which developed in the untreated fish. It also inhibited a rise in TBA values in both ordinary and red muscle (Table I). In the untreated samples, TBA values rose to quite high levels, but the increase occurred only after the limit of acceptability (about 8 days) was reached, indicating that oxidative rancidity did not contribute appreciably to development of the off-odors and flavors responsible for rejection by the taste panel.

DISCUSSION

In the muscle of mackerel iced either immediately after catching or after 6–8 hr as in commercial practice, postmortem dephosphorylation of IMP proceeded much more slowly (Hx not yet maximum at 20 days) than in muscle of iced trawled gadoids (Jones, 1965b) and of certain Pacific coast soles and rockfish (Spinelli et al., 1964) where peak levels of Hx were reached at 10–16 days. The rate, however, was not as slow as in iced swordfish (Dyer et al., 1966), where Hx had increased to levels of only 1 μmole/g at 19 days. These findings provide further support for earlier observations (Kassemsarn et al., 1963) that the activities of the enzymes responsible for nucleotide catabolism vary appreciably with species.

The *p*H of optimal activity of the nucleotidase and nucleosidase enzymes involved is not well known. In lingcod, riboside hydrolases exhibited a maximum peak of activity at *p*H 8.6, with a minor one at *p*H 5.5 (Tarr, 1966). Nucleotidases have been little studied in fish, but in general have a *p*H optimum on the alkaline side of neutrality (Laskowski, 1951). Hence the relatively low initial *p*H in mackerel, <6.2, and in swordfish, 5.7–6.1 (Dyer et al., 1966), may be an important factor in the slow initial rate of nucleotide breakdown in these species. Also, the change in *p*H from 6.3 to 7 observed in the stored mackerel may result in some change in enzyme activity over the storage period.

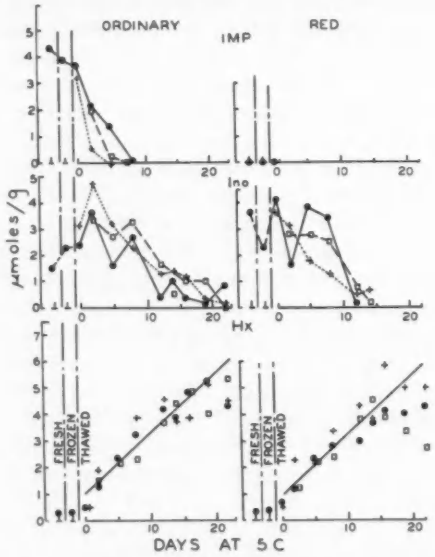

FIG. 3. Nucleotide degradation in ordinary and red muscle of thawed mackerel during storage at 5 C. The fish, held at ambient temperatures before processing, had been quick-frozen and stored 6 weeks at −26 C before thawing (expt. C); ●——● thawed slowly in air at 5 C; □- - -□ thawed slowly in air at 5 C, dipped in 0.5% ascorbic acid before freezing; +.....+ thawed rapidly in water at 18 C. Regression lines are shown for Hx; since there were no apparent differences between the three treatments, all points to 16 days were combined for calculation.

	Regression equation	$\bar{x}$ (*days*)	$\bar{y}$ (*μmoles/g*)	Goodness of fit[a] (*t*)	95% confidence interval of mean at midpoint	*n*
Hx (linear)						
Ordinary	Y = 0.23X+0.98	8.56	2.97	11.5***	±0.23	20
Red	Y = 0.23X+0.98	8.56	2.91	9.7***	±0.26	20
Hx (log [Hx times 100] vs. time)						
Ordinary	Y = 0.046X+2.00	8.56	2.40	7.5***	±0.070	20
Red	Y = 0.042X+2.04	8.56	2.40	8.4***	±0.057	20

[a]See footnote a, Fig. 1.

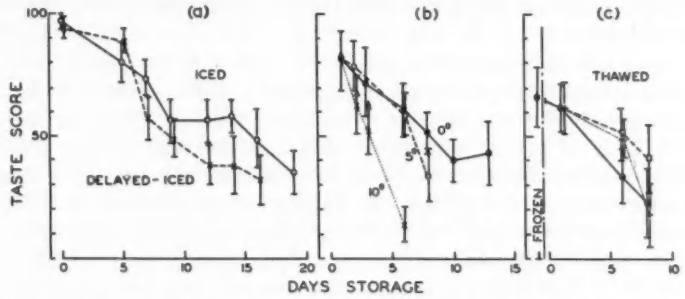

FIG. 4. Taste scores of mackerel. Mean scores at each sampling period with 95% confidence intervals are shown (number of samples was 23 in expt. A and 16 in expt. B and C). (*a*) iced immediately after catching (○———○) and after 7 hr delay at 13–20 C (×———×) (expt. A); (*b*) held at 0 (●———●), 5 (○- - -○), 10 (×.....×), and 15 C (△-·-·-·△) (expt. B); (*c*) held at 5 C after thawing: in air at 5 C (●———●), in water at 18 C (+.....+), and in air at 5 C of fish dipped in 0.5% ascorbic acid before freezing (□ - - - □) (expt. C).

TABLE I. TBA values (OD at 538 mμ/g of muscle) in ordinary and red muscle of mackerel during storage at 5 C after slow thawing at 5 C and rapid thawing at 18 C. Frozen mackerel stored at –26 C for 6 weeks before thawing.

Days storage at 5 C after thawing	Ordinary muscle[a]			Red muscle		
	Thawed at 18 C	Thawed at 5 C	Dipped[b] thawed at 5 C	Thawed at 18 C	Thawed at 5 C	Dipped[b] thawed at 5 C
5	0.9	0.7	1.3	1.8	1.3	1.6
12[c]	0.5	1.7	0.5	0.9	1.8	1.3
19	2.3	2.3	0.9	2.8	2.9	1.3
26	2.3	2.9	0.7	2.2	3.1	1.3

[a]TBA value in the ordinary muscle before freezing was 0.5.
[b]Dipped in ascorbic acid before freezing.
[c]The fish were judged unacceptable at 8 days.

In well-handled mackerel iced from the moment of capture, eating quality was maintained to 19 days, similar to well-iced, dressed swordfish (Dyer et al., 1966) and considerably longer than the 12–14 days usually encountered in trawled cod and other whitefish (Reay and Shewan, 1949). A contributing factor to this extended keeping time may possibly be the trend to a more acid flesh in iced mackerel, indicated by a higher lactate content and lower *p*H than in whitefish species (Cutting, 1953).

Effect on Quality of Delayed Icing, Thawing, and Antioxidant Treatment

Delayed icing for about 7 hr promoted a more rapid spoilage rate during subsequent storage in ice than in continuously iced fish. This was shown both by differences ($P < 0.05$) in taste scores at 12–14 days (Fig. 4*a*) and by the regression lines (Fig. 5). The latter illustrate the significantly more rapid deterioration rate and lower level of taste scores with delayed icing. This might be expected in view of the very rapid changes observed in the fish held at elevated temperatures (15 and 20 C); however, no statistically significant differences in IMP dephosphorylation or Hx formation were found, although an earlier IMP dephosphorylation in the delayed iced fish is indicated.

During frozen storage at −26 C for 6 weeks and during thawing (expt. C), little change occurred in IMP (Ino increased slightly). In swordfish, we had observed previously no change in nucleotide levels during 4–5 weeks storage at −26 C (Dyer et al., 1966). Similarly, Jones (1965b) found the IMP-dephosphorylating enzyme (5′-nucleotidase) inactive in cod during plate freezing, storage at −30 C, and also during slow thawing. During subsequent storage of slowly thawed mackerel at 5 C, the rate of IMP dephosphorylation was almost the same as in the unfrozen samples stored at 5 C (expt. B). However, two separate lots of fish were used and the IMP level in the thawed fish at commencement of storage at 5 C was only about half that of the unfrozen lot; this accounts for the earlier depletion of IMP as well as the earlier peaking of Ino in the thawed lot. Hx formation in the thawed fish (Fig. 3) appeared to occur considerably more rapidly in the early stages (to 15 days) in contrast to the unthawed fish; slopes of the regression lines are 0.23 and 0.15 respectively, and this may indicate enzyme activation by the thawing process. This point should be further investigated using both unfrozen and thawed fish from the same lot. Overall grade taste panel scores indicate that thawing did not affect deterioration rates during subsequent storage at 5 C.

Ascorbic acid was effective in preventing darkening of the flesh and rancidity as measured by TBA value, but had little effect on organoleptic quality, since most of the inhibition occurred after the limit of unacceptability had been reached.

Nucleotide Catabolism in the Red Muscle

In red muscle of mackerel, the pattern of nucleotide decomposition appeared similar to that in the ordinary muscle, as previously reported for swordfish (Dyer et al., 1966). With all lots of mackerel investigated, the sequence of nucleotide changes was more advanced in the red than in the ordinary muscle at the time of first sampling; IMP levels in the red had been reduced to 0–2 μmoles/g and initial Ino levels were correspondingly higher. Since total adenosine and inosine compounds were only slightly lower in the red muscle, 6.6 ± 0.1 μmoles/g compared with 8.0 ± 0.1 for the ordinary muscle (84 samples each), this indicated a greater 5′-nucleotidase activity in the red muscle during the antemortem struggle and/or the subsequent storage

up to 6–8 hr prior to sampling. The present results indicate that, in the rather active mackerel containing a high proportion of red muscle, high-energy phosphate compounds were depleted ante-mortem in both red and ordinary muscle; in contrast, in the red muscle of exhausted cod, only partial depletion of the high-energy nucleotides occurred ante-mortem (Fraser et al., 1966). In contrast to the apparent more active antemortem nucleotidase in the red muscle noted above, the postmortem rates of Hx accumulation (regression line slopes) indicated that the activity of the nucleosidase responsible for the degradation of Ino to Hx was similar in the red and ordinary muscle under the various experimental conditions used (Fig. 1, 2, and 3).

Temperature and Postmortem Nucleotide Degradation and Quality

The results demonstrate the marked effect of temperature, over the range 0–20 C, on the changes in nucleotides and quality. Such data, covering temperatures likely to be encountered in commercial practice, have hitherto been scarce. With respect to the relationship between Hx and time, inspection of the data and of the linear regression lines (Fig. 2, as well as Fig. 1 and 3) indicates that this relationship may sometimes be slightly curvilinear rather than linear. The linear relationship, however, was highly significant when the "goodness of fit 't' test" was applied, P being <0.001 in 10 out of 14 cases for red and ordinary muscle and not exceeding 0.005. The linear regression line gives a better fit than a logarithmic relationship (values tabulated in Fig. 1, 2, and 3), whereas log–log or inverse relationships did not yield significant results.

In some cases, for instance after 15 days at 0 and 5 C in Fig. 2, there is an apparent change (increase) in the rate of Hx accumulation, which probably indicates a change in the mechanism of the reaction. This could result from the additional participation of bacterial enzymes at this point, as suggested previously (Jones, 1965a; Dyer et al., 1966), although Spinelli's results (1965) on radiation-pasteurized sole favor the view that endogenous enzymes alone were mainly responsible for Ino degradation. It may also result from the increase in pH which occurred as spoilage advanced, causing changes in the enzyme activity, as noted above.

Taste in Relation to Nucleotide Changes

As with swordfish, cod, and related species (Dyer et al., 1966; Jones, 1965a), a considerable portion of the nucleotide degradative sequence in mackerel was completed during the period of edibility. For the various lots of fish and handling conditions studied, the dephosphorylation of IMP was complete, or nearly so, by the time the limit of acceptability was reached; also, decrease in taste scores closely paralleled the loss of IMP (see next section) from the ordinary muscle. A progressive loss of "fresh mackerel" flavor leading to tastelessness, evident before the appearance of offensive bacterial spoilage odors and flavors, could be attributed to the concomitant loss of IMP, widely

recognized as a major contributor to the desirable flavor of fresh fish (e.g. Jones, 1961; Hashimoto, 1965).

In the present work, the mackerel was rejected by the taste panel prior to the formation of appreciable quantities of Hx (at levels of 1.2–2.5 μmoles/g). At this point, only occasionally were traces of bitterness noted by a few panelists; common bacterial spoilage odors and flavors, described as "stale," "fishy," and "rancid" predominated at this time. Although these Hx levels are sufficiently high to impart bitter flavors (Jones, 1961), the possible contribution of Hx to the overall organoleptic complex in spoiling fish is apparently subtle and as yet imperfectly understood (Jones et al., 1964; Spinelli, 1965; Hughes and Jones, 1966).

IMP, Hx, and Ino + Hx as Indices of Quality

Any quality index is most useful when it can be applied to a variety of handling conditions and species. Most previous work concerning the variability of IMP, Hx, and of the Ino + Hx fraction as quality indices was conducted at ice temperature only. The present data on mackerel extend these studies to include various other handling conditions such as holding at temperatures between 0 and 20 C, and thawing.

Regression lines of taste scores on contents of IMP and Hx (Fig. 6) show highly significant ($P < 0.001$) linear relations, the 95% confidence limits at the midpoint showing variations in taste score of ±4.3 and ±6.4 respectively. It should be noted that IMP has decreased to 0 at an average taste score of 41, i.e. at the limit of acceptability. Thus, the absence of IMP in a sample

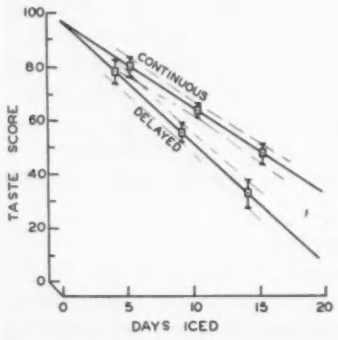

FIG. 5. Regression lines for taste scores vs. time in ice for continuously iced and delayed iced mackerel (expt. A). At each sampling time there were 23 judgments for the continuously iced and 15 for the delayed iced. 95% confidence intervals of the mean are shown: for continuously iced, Y = 97–3.2X (n = 181); for delayed iced, Y = 97–4.5X (n = 105).

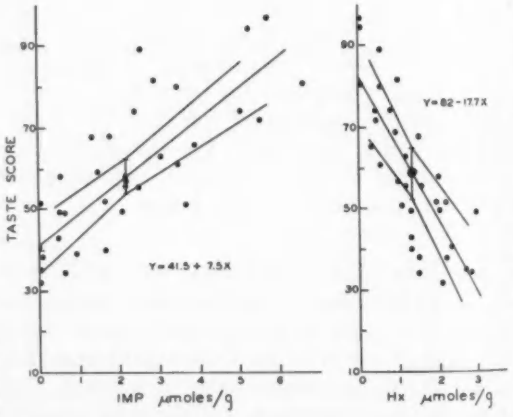

FIG. 6. Regression lines of taste scores on contents of IMP and of Hx. The points include data on mackerel iced immediately and after a delay of 6–8 hr; held at 0, 5, 10, and 15 C; and thawed mackerel held at 5 C (expt. A, B, and C). 95% confidence intervals of the mean are shown.

indicates a quality at best either at or near the unacceptability level. Also, a Hx content of 2.5 μmoles/g is coincident with a taste score level of 40 (95% confidence interval of the mean is 30–50). Unlike IMP, Hx formation continues beyond the unacceptability level and thus may be used as a confirmatory test of spoilage, as suggested previously (Dyer et al., 1966).

The simpler measurements of combined Ino + Hx resulting from IMP degradation were examined (Fig. 7). Both the direct ultraviolet absorption

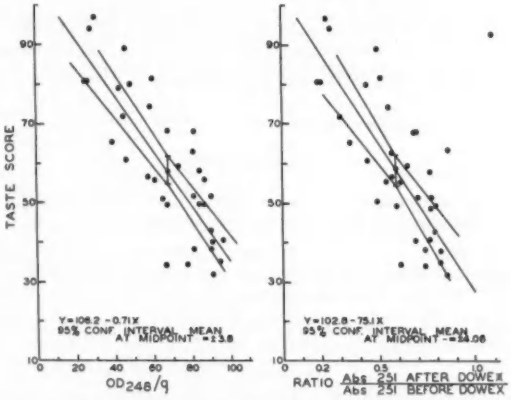

FIG. 7. Regression lines of taste scores on OD_{248} of Dowex-treated $HClO_4$ extracts, and on ratio of ultraviolet absorption at 251 mμ after to that before shaking extracts with Dowex. Samples as in Fig. 6. 95% confidence intervals of the mean are shown.

at 248 mμ (OD_{248}/g fish) of neutralized perchloric acid extracts after shaking with Dowex and the ratio of the ultraviolet absorption at 251 mμ of such extracts after shaking with Dowex to that before shaking showed highly significant linear relations with taste scores. Both indices yield equally good predictions of taste scores, e.g. at the limit of acceptability the 95% confidence intervals range from 35 to 45. Since the OD_{248} procedure is simpler in that it involves only one spectrophotometric measurement, use of this method is to be preferred as a quality index. The OD_{248} is indeed a true measure of Ino + Hx in mackerel, since column chromatographic separation and chemical determination showed that Ino and Hx accounted for 95% of the purine compounds not removed from neutralized perchloric acid extracts by shaking with Dowex. On the other hand, with the ratio index method, accurate measurement of weights of fish muscle and of volumes of extractant are unnecessary; it also allows for possible greater variation in levels of total purine compounds in species other than mackerel. We agree with Jones (1965a) that for routine use as a freshness index the time-consuming column chromatographic separation of the Ino + Hx fraction (Saito et al., 1959; Fujii et al., 1966) is unnecessary,

both because of the excellent correlation of quality with both the OD_{248} and ratio indices found in the present experiment, and because of the high purity of the fraction not absorbed by Dowex. With untried species, it should be established that the pattern of nucleotide degradation is similar.

ACKNOWLEDGMENTS

We wish to thank Mr C. A. Campbell for his excellent cooperation in obtaining and in processing the fish used.

We also thank A. Cohen, H. C. Freeman, R. J. Hoyle, M. McMenemy, M. Morton, W. Neal, S. Nowlan, P. H. Odense, H. Power, J. Simmonds, R. Sinclair, and A. L. Wood, who willingly served on the taste panels in addition to the authors.

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Marking Small Fish with Stains for Estimating Populations in Heming Lake, Manitoba[1]

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ABSTRACT

For estimating populations of trout-perch, *Percopsis omiscomaycus* (Walbaum), by immersing fish in solutions of stains, Bismarck brown Y was superior to two other stains tested. It was satisfactory for periods of marking up to 1 week. Neutral red indicator can be used in marking experiments of less than 2 days' duration but Nile blue A faded rapidly and is not recommended for use in estimating populations. Using multiple and single census methods of estimating population size, the most reliable estimates of trout-perch greater than 46 mm in length were 711,451 (Schnabel) and 883,148 (Petersen), or in number per acre 1186–1472.

INTRODUCTION

This paper describes the experimental testing of three stains as possible means of marking fish for tag and recapture studies; two, Bismarck brown Y and Nile blue A, were certified biological stains, and the third was an indicator solution of neutral red rather than the certified biological stain. After experimental testing these stains were used to mass mark trout-perch, *Percopsis omiscomaycus*, an important forage fish in Heming Lake, whose small size did not permit the use of conventional fish tags. Schnabel and Petersen types of population estimates were derived from the recapture of stained (marked) trout-perch which subsequently appeared in trawl catches along with unstained trout-perch.

Non-mutilation marking methods have been fully reviewed by Gerking (1963); these include (1) total immersion in a biological stain; (2) tattooing; (3) injection of various substances; and (4) marking with radioactive isotopes. He concluded that the most advantageous method for marking small fish for short-term experiments is by immersion in an appropriately diluted solution of a biological stain.

Of the several biological stains tested by Deacon (1961), Bismarck brown Y gave the best results and was used on several species of fish but the trout-perch was not one of those tested. At concentrations of 1:5000 at 18 C or 1:20,000 at 29 C for 3 hr, he found that the fish became brightly coloured and remained recognizable for 1–2 weeks. Ward and Verhoven (1963) used neutral red and Bismarck brown Y to stain sockeye salmon fry. Initial mortalities were low in experiments using neutral red but all fry, regardless of concentration or immersion time, were dead on the 4th day; unstained fish survived well.

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J. Fish. Res. Bd. Canada, 25(2): 255–266, 1968.
Printed in Canada.

Concentrations of 1:5000 and 1:10,000 of Bismarck brown Y in the range used on non-salmonid species by Deacon (1961) were almost immediately lethal to sockeye salmon fry when tested by Ward and Verhoven (1963). They concluded that Bismarck brown Y was suitable for marking sockeye salmon in experiments of approximately 7 days' duration when the concentration and immersion times were as follows: 1:30,000 for 1½ hr; 1:60,000 for 3 hr; or 1:120,000 for 3 hr.

Since 1961 a tag and recapture program has been carried out at Heming Lake, Manitoba, to compare under field conditions the statistical techniques that have been developed for the estimation of fish populations which cannot be measured by direct total count or census. Also included will be the estimation of other parameters of population dynamics such as recruitment rate, growth rate, mortality rate, and exploitation rate. Until 1966 no attempt had been made to mark fish, of any species, less than 12½ cm in length. Before applying staining techniques to the small fish of the major species being tagged (white sucker, northern pike, yellow perch, walleye, and burbot), it was felt desirable to attempt to mark species of smaller size, such as minnows, which are present in considerable numbers. The trout-perch, *P. omiscomaycus*, which is taken in large numbers in the various types of fishing gear, is one of the main food species in the diet of the pike of Heming Lake (Lawler, 1965) but up to the start of this experiment was not tagged because of its small size. From mass marking with these stains, Schnabel and Petersen types of population estimates were derived from data on ratio of stained to unstained trout-perch recorded from trawl catches.

MATERIALS AND GENERAL METHODS

A semi-balloon trawl net of ½-inch stretched mesh in the cod-end was used to capture all trout-perch used in this study. Tows were of 3–5 min duration to ensure that all fish to be handled were in the best possible physical condition. Trawling was carried out along the length (5.6 km) of the lake in water depths ranging from 2.7 to 5.5 m. The mean length of the trout-perch was 63 ± 3.0 mm and the range in size was from 46 to 103 mm.

The staining agents were each weighed on a precision balance and dissolved in lake water to the required concentration. The Bismarck brown Y contained 47% dye and Nile blue A 91%. The dye solution was held in square plastic tubs each containing about 10 gal and to these the samples of the fish to be marked were introduced, usually about 100–200 fish per tub. The tubs were aerated by means of a small air compressor and after the required time in the stain the fish were then transferred to holding troughs until they had recovered sufficiently to be returned to the lake, or alternatively they were transferred to 5-gal aquaria to determine mortality rates and observe colour changes. These aquaria were supplied with a continuous flow of lake water. Continuous temperature records were kept as the temperature in the aquaria was more subject to sudden fluctuations than water in the lake because the water flow was reduced at night and external temperatures decreased markedly during the

cool August nights. The number of stained fish released into the lake for subsequent recapture was restricted to apparently healthy individuals.

Stained trout-perch were placed in the lake and subsequently recaptured in trawls to estimate populations and to determine how long these fish might still be recognizable (Table I).

Colour photographs of stained fish from the aquaria and from the lake catches were taken regularly to record colour changes as they occurred.

TABLE I. Recaptures of trout-perch marked with three stains.

Day	No. of recaptures			No. of trawling efforts
	Bismarck brown Y	Neutral red	Nile blue A	
Aug. 8				–
9	5			21
10	11			20
11[a]	20			19
12	93			36
15	35			14
16	6			6
17	8			3
18	0			2
22[b]				29
23	2	12	11	39
24	2	5	0	18
25	0	0	0	2
26	0	0	1	3
29	3	0	0	4
30	5	1	1	4
31	6	2	0	4
Sept. 5	1			4
6	3			4
7	2			4
8	1			2

[a]Bismarck brown Y staining terminated this date.
[b]Neutral red and Nile blue A staining terminated this date.

TESTS OF STAINING AGENTS

BISMARCK BROWN Y

Initial attempts to stain trout-perch were made at a concentration of 1:6000 for 3 hr at 20 C. Mortalities at this concentration were extremely high and none of the stained fish were released. Autopsies performed immediately after the fish were removed from the staining bath showed that the stain had permeated the musculature and viscera, and had even penetrated the vertebral column.

Mortality was lower for trout-perch tested at 1:12,000 for 2 hr than it had been at the previous concentration. At a dosage of 1:24,000 and a staining time of 2 hr the mortality was still lower. This concentration was used for the first 3 days to mark fish for purposes of estimating population size. On the 4th and final day of marking the concentration of the stain was increased to 1:12,000 but the staining time was reduced to 1 hr. Although the mortalities were known to be slightly higher than they had been at 1:24,000 it was possible to mark more fish per day and it was felt this offset the disadvantages of slightly increased mortality. Staining trout-perch with Bismarck brown Y for population estimation began August 8 and was completed August 11.

Some trout-perch stained with Bismarck brown Y were retained in aquaria to observe changes in colour with time but no controls were kept to investigate differences in mortality between marked and unmarked fish. However, it should be noted that after 115 hr in the aquaria the mortality of these trout-perch was 100% (Fig. 1). This mortality was likely due to the adverse effects of fluctuations in temperature within the aquaria rather than the toxic effects of the stain.

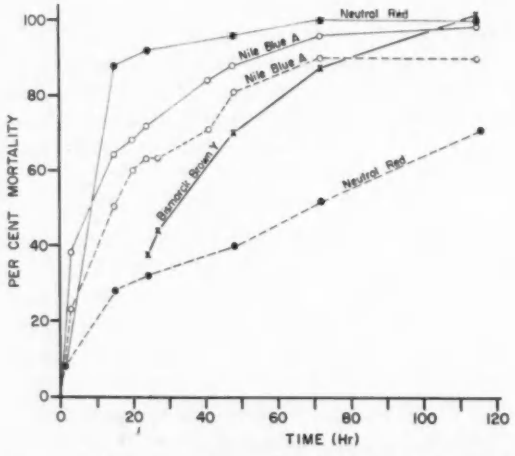

FIG. 1. Mortalities in aquaria of trout-perch stained with Bismarck brown Y, Nile blue A, and neutral red indicator (solid lines) and of fish not stained (broken lines) (no control data for fish stained with Bismarck brown Y).

On the basis of information in the literature, marking was discontinued after 4 days to ensure that population estimates would not be biased by missing stained fish that had faded within the period that fish were being marked and recaptured. Marking and recapturing might have been extended over a longer period as a few fish were still recognizable as having been stained with Bismarck brown Y 28 days after the marking had been completed (Table I).

Although the number of tows made after completion of the sampling for population estimates was small, the return of marked fish was higher than had been anticipated.

Nile Blue A

Two series of experiments were carried out in testing Nile blue A as a staining agent as the first tests did not yield satisfactory results. In the first series fish were immersed in concentrations of 1:5000; 1:10,000; 1:15,000; and 1:25,000 for 1 hr. The temperatures in the staining baths were between 15 and 15.5 C. Temperatures in the holding aquaria, however, varied as much as 5 degrees C in one 24-hr period as a result of changes in the air temperature particularly at night.

At a concentration of 1:5000 for 1 hr, mortalities were extremely high; indeed more than 90% were dead on removal from the staining bath. After 3 hr the remaining fish from the sample were dead. Autopsies performed after the fish had been removed from the stain indicated a heavy deposit of dye on the gills and gill membranes and dye penetration of the epidermis of the dorsal and lateral surfaces of the body. On the ventral surface the dye had penetrated the musculature.

Mortalities were much lower at a concentration of 1:10,000 as only 2 of the 30 stained fish were dead on removal from the stain. After 1 hr these stained fish were then placed in a holding aquarium and 7 fish were dead. After 24 hr 26 of the fish had died and at the end of 48 hr only 1 stained fish remained alive. This fish appeared healthy, but the stain had faded considerably and was recognizable only in the ventral region.

Mortalities in the holding aquarium were even lower at 1:15,000 than in the 1:10,000 concentration. The ventral region of the body was well stained. Little apparent difference in appearance was noted between the fish stained at this concentration and those stained at the 1:10,000 concentration; however, no heavy deposits of stain were noted in the gill region as had been observed on fish stained in the 1:5000 concentration.

At a concentration of 1:25,000, mortalities were approximately the same as with the previous concentration. On dissection only the ventral musculature appeared to have absorbed the stain. Although all fish in the holding aquarium had died 40 hr after staining, the last to die was still recognizable as a stained fish.

As in the second series of tests with Nile blue A the higher concentrations were not used but at the lower concentrations the results were very similar to those for the first test. A concentration of 1:30,000, not used in the first series, was tested and after 24 hr in a holding aquarium the fish that had been stained were almost unrecognizable. At this concentration some fish in the aquarium were still alive after 111 hr.

Although Nile blue A may have some toxic effect, widely fluctuating temperatures in the aquaria made it rather difficult to isolate the real cause of mortalities.

For marking purposes a concentration of 1:25,000 was used. The fish were stained August 22 and recaptured August 23 and 24. The greatest number of recaptures (11) was recorded the day after marking but stained fish were still evident in the catch 8 days after marking (Table I). Fading was evident in fish caught 3 days after staining, the colour being most apparent on the fins and around the mandible.

Brightly stained fish may attract predators, as trout-perch stained with Nile blue A were found in pike stomachs 2 days after staining. This observation is in general agreement with the findings of Lawler and Smith (1963) who reported that for small fish the return of opaque yellow tags was significantly smaller than that of clear transparent tags (less than one-half) and interpreted this as due to a higher predation rate.

Neutral Red Indicator Solution

Trout-perch were immersed in baths of neutral red indicator solution at concentrations of 1:30,000, 1:20,000, and 1:10,000 for 1 hr at a temperature of 17 C. A sample which had not been stained was retained for control purposes.

The intensity of colour in the stained fish was proportional to the concentration of the stain used. At a concentration of 1:10,000 the stain had permeated the musculature but not the viscera. At this concentration the stain appeared to leave a deposit on the gills. Although the whole fish was stained red, the dye seemed more pronounced on the gill region, operculum, and ventral fins. At concentrations of 1:20,000 and 1:30,000 the stain was also apparent in the musculature; in the gill region no deposit was observed at the 1:30,000 concentration. Mortalities were slightly higher at concentrations of 1:20,000 and 1:30,000 than in the higher concentration of 1:10,000. After 3 days all fish in the aquaria had begun to lose their stain and were becoming difficult to identify.

For marking and recapture the 1:30,000 concentration was used as there appeared to be sufficient retention of stain to enable the program to be carried out.

Trout-perch stained with neutral red indicator solution were captured in trawls up to 9 days after marking (Table I).

POPULATION ESTIMATES

Data obtained from the marking experiments, conducted at Heming Lake during the summer of 1966, provided the basis for independent estimates of the population size of the trout-perch exceeding 46 mm total length. Estimates of the Schnabel multiple census and Petersen single census types were conveniently made. These estimates and their associated 95% confidence ranges were derived from formulae and charts appearing in Ricker (1958). It was not possible to use the DeLury catch per unit effort method of population estimation because of the design of the marking experiments.

Schnabel Type Estimates

The data obtained from the continuous marking–recapture program using Bismarck brown Y stain to mark the fish are given in Table II. A total of

TABLE II. Marking, recapture, and computational data for multiple census estimates of the Heming Lake trout-perch population marked by staining in Bismarck brown Y.

Date	No. of fish caught (C_t)	No. of recaptures (R_t)	No. of fish stained	No. of fish released (at end of each day)	No. of stained fish at large (M_t)	No. of trawling efforts	$C_t\ M_t$	$M_t\ R_t$	$C_t\ M_t^2$	$\frac{R_t^2}{C_t}$
Aug. 8[a]	1,525	0	1,525	1,394[b]	0	13				
9	2,230	5	2,225	2,157	1,394	21	3,108,620	6,970	4,333,416,280	0.01121
10	2,391	11	2,380	2,346	3,551	20	8,490,441	39,061	30,149,555,991	0.05061
11[c]	3,148	20	3,128	2,992	5,897	19	18,563,756	117,940	109,470,469,132	0.12706
12	7,098	93	–	–	8,889	36	63,094,122	826,677	560,843,650,458	1.21851
15	3,714	35	–	–	8,883[d]	14	32,991,462	310,905	293,063,156,946	0.32985
Total:	–	164	9,258	8,889	–	123	126,248,401	1,301,553	997,860,248,807	1,73724

[a]Initial day of staining.
[b]On August 8, 131 marked trout-perch were removed (i) for use as controls (ii) because of staining mortality.
[c]Final day of staining.
[d]This figure incorporates known losses of six marked trout-perch.

8889 stained fish were released between August 8 and 11. On the 5th day of the experiment (August 12) four stained trout-perch were found amongst the stomach contents of autopsied pike, *Esox lucius*, and two others were found dead on the shore of the lake. An appropriate amendment was therefore made to the number of marked fish at large (Table II).

The validity of Schnabel estimates is known to be dependent upon three important factors affecting the constancy of the population during the experiment. The first consideration is that there is no recruitment to the population throughout the period of sampling. Due to the brevity of the experiment (7 days) this variable was considered to be effectively eliminated. Secondly, there should be no mortality to the population. There was some indication that marked fish may experience a slightly higher percentage mortality upon release than unmarked fish. However, for consistency, an arbitrary staining-mortality figure was not incorporated into the calculations. Finally, the population should be randomly sampled. Although this factor can never be proven, it may be assumed with confidence by virtue of the mode of fishing. The stained fish were returned to the precise location from which they were trawled and at least 12 hr were allowed to elapse between consecutive tows in any one location.

The original Schnabel (1938) estimates, the modifications suggested by both Chapman (1952, 1954) and Schumacher and Eschmeyer (1943), together with their respective 95% confidence limits, are presented in Table III. These estimates were derived from 4 days (August 9, 10, 11, and 12) and 5 days (August 9, 10, 11, 12, and 15) of recapture data respectively. The differences between the corresponding estimates for these two periods are not appreciable. Also the number of recaptures per unit of trawling effort for August 12 and 15 give approximately the same value of 0.25. These data indicate that the majority of the 8883 marked fish extant were still retaining their stain at least 4 days after the final day of staining. However, since the Schnabel estimate tends to increase with a proportional decrease in the number of recaptures, it is inferred that a small percentage of the marked fish had become unidentifiable at some time during the sampling period. The preferred multiple census type of estimate has been shown by DeLury (1951) to be that based upon the Schumacher and Eschmeyer formula. The values obtained by this method from the present data for 4 days of recaptures was 711,451 and for 5 days of recaptures 766,669.

Petersen Type Estimates

A single day of marking, using approximately equal numbers of trout-perch stained with Nile blue A and neutral red indicator solution, together with 2 subsequent days of recaptures afforded the data (Table IV) for computing Petersen estimates of the population size. It is to be noted that the fish suffered a 10% staining mortality in both stains and as mentioned previously only healthy-looking stained fish were released. Also, a correction was made

TABLE III. Multiple census estimates of the trout-perch population size from marking with Bismacrk brown Y.

Type of estimate	No. of days of recapture data	Population estimate ($\hat{N}$)	95% confidence range
Schnabel[a]	4	722,922	616,493–873,765[d]
Chapman's modification[b]	4	717,362	612,163–866,217[e]
Schumacher and Eschmeyer modification[c]	4	711,451	621,770–831–083[f]
Schnabel	5	769,807	667,628–908,916
Chapman's modification	5	765,142	663,871–902,871
Schumacher and Eschmeyer modification	5	766,669	651,432–931,438

[a]Given by $\hat{N} = \frac{\Sigma C_t M_t}{\Sigma R_t}$.

[b]Given by $\hat{N} = \frac{\Sigma C_t M_t}{(\Sigma R_t)+1}$.

[c]Given by $\left(\frac{\hat{1}}{N}\right) = \frac{\Sigma M_t R_t}{\Sigma(C_t M_t^2)}$.

[d]Using var $\left(\frac{\hat{1}}{N}\right) = \frac{R}{(\Sigma C_t M_t)^2}$.

[e]Using var $\left(\frac{\hat{1}}{N}\right) = \frac{R+1}{(\Sigma C_t M_t)^2}$.

[f]Using standard error $\left(\frac{\hat{1}}{N}\right) = \frac{s}{\sqrt{\Sigma C_t M_t^2}}$ where $s^2 = \frac{\Sigma(R_t^2/C_t) - (\Sigma R_t M_t)^2/\Sigma C_t M_t^2}{m-1}$

and m is the number of sampling days.

to the number of fish at large, because of the occurrence of three stained fish (one red, two blue) in the stomachs of autopsied pike.

A prerequisite for the validity of Petersen estimates is that the proportion of marked fish present in the population remains constant throughout the sampling. It is clear that during the short period of sampling (3 days) the amount of recruitment to the population may be considered negligible. Hence, the only major factors which will affect the ratio of the number of marked fish to the total number of fish during the experiment are: (1) differential mortality between marked and unmarked fish; (2) the inability to recognize a "marked" individual because of the fading or complete loss of its stain. Although unstained fish may have a slightly lower mortality than stained fish, an arbitrary compensation factor was not included in the data of Table IV. Furthermore, the recapture data clearly indicate that during the sampling period fewer Nile blue A stained fish than the neutral red stained fish were being identified by the fishing crews. Indeed, on August 23, for the 5663 trout-perch sampled, the ratio of neutral red to Nile blue A recaptures was 12:11,

TABLE IV. Marking and recapture data for single census estimates of the trout-perch population size from marking with neutral red and Nile blue A.

Date	No. of fish caught (C)	No. of stained fish released	Percentage staining mortality	No. of stained fish at large (M)	No. of recaptures (R)	No. of trawling efforts
			Neutral red			
Aug. 22	2256	2028	10.1	–	–	29
23	5663	–	–	2027[a]	12	39
24	3574	–	–	2027	5	18
Total (Aug. 23 × 24)	9237	–	–	2027	17	57
			Nile blue A			
Aug. 22	2516	2266	9.9	–	–	29
23	5663	–	–	2266	11	39
24	3574	–	–	2264[a]	0	18
Total (Aug. 23 × 24)	9237	–	–	2264	11	57
			Neutral red and Nile blue A			
Aug. 22	4772	4294	10.0	–	–	29
23	5663	–	–	4293	23	39
24	3574	–	–	4291[a]	5	18
Total (Aug. 23 × 24)	9237	–	–	4291	28	57

[a]This figure incorporates known losses from the stomach contents of autopsied pike.

even though more blue-stained than red-stained fish had been released the previous day. The equivalent ratio for the 2nd day of recaptures (August 24) was 5:0, the number caught being 3574. Because of the relatively low number of recaptures, values for catch-per-unit-effort should be viewed with some reservation; however, it should be noted that for the 1st and 2nd days of recaptures there was a marked difference between the Nile blue data (0.30–0.00%) and the neutral red data (0.30–0.27%). The superiority of the neutral red to the Nile blue A stain for short-term marking and recapture experiments is, however, clearly apparent.

A further condition should be imposed upon the sample sizes M (number of fish marked) and C (number of fish recaptured) in order to ensure a valid Petersen estimate (Robson and Regier, 1964). They established that the product of M and C must exceed four times the guessed order of magnitude of the population size (N). Also they include graphs to determine the sample sizes M and C for the guessed values of N at various levels of precision and accuracy. For population dynamics research, the highest level of $1 - \alpha = 0.95$ and $p = 0.10$ are recommended; that is, errors in population size of 10% are acceptable to the investigator if there is a 95% certainty that his error will not exceed this 10% value. Based upon the judicial guess of 500,000 (N) for

the trout-perch population of Heming Lake, it is apparent that the product MC for the Nile blue A, neutral red, and combined data (Table IV) well exceed 2,000,000 (4N) as required. At the highest level of accuracy it is observed that, with the same assumed value of N, the number of fish examined for stain (C) should have somewhat exceeded 9237 for the number marked (M) of 4291.

From the viewpoint of population estimates (Table V), the Nile blue A data alone are considered to be of dubious value. Likewise, the incorporation of the Nile blue A data into the combined recapture data tends to confound

TABLE V. Single census estimates of the trout-perch population size from marking with neutral red and Nile blue A.

No. of days of recaptures	Type of stain	Type of estimate	Population estimate	95% confidence range[a]
1	Neutral red	Petersen[b]	956,575	573,945–1,913,150
2	"	Petersen	1,101,376	693,459–1,970,884
1	"	Bailey's modification[c]	883,148	673,149–1,640,132
2	"	Bailey's modification	1,040,301	668,765–1,783,374
1	Nile blue A	Petersen	1,166,578	658,070–2,566,471
2	"	Petersen	1,901,142	1,072,439–4,182,514
1	"	Bailey's modification	1,069,552	626,079–2,139,104
2	"	Bailey's modification	1,742,903	1,020,236–3 485,805
1	Neutral red and Nile blue A	Petersen	1,057,011	694,607–1,620,751
2	"	Petersen	1,415,570	966,731–2,086,103
1	"	Bailey's modification	1,013,148	675,432–1,519,722
2	"	Bailey's modification	1,366,905	943,816–1,982,013

[a]Treating R as a Poisson variable and using Adam's (1951) chart.

[b]Using $\hat{N} = \frac{MC}{R}$.

[c]Using $\hat{N} = \frac{M(C+1)}{R+1}$.

the population size results. The most reliable estimates for the present data are therefore considered to be those based upon 1 and 2 days of recaptures of neutral red stained trout-perch alone. Bailey's (1951) modified formula, which gave an estimate of 883,148 for 1 day of recaptures (1,040,301 for 2 days), is generally favoured over the original Petersen (1896) formula, which yielded 956,575 for 1 day of recaptures (1,101,376 for 2 days).

CONCLUSIONS

Since different stains were used in the two marking experiments, it would be erroneous to make a direct comparison of the Schnabel and Petersen estimates computed above. As a result it is suggested that a truly meaningful

comparison of these estimates could only be made if further data were available from an experiment of the single census (Petersen) type using Bismarck brown Y as a staining agent.

For periods of marking up to a week, Bismarck brown Y stain was far superior to the other stains tested. The neutral red stain only appears to be satisfactory for short-term use in experiments of less than 2 days' duration. Nile blue A rapidly fades under experimental conditions and is therefore not recommended for total immersion marking procedures in which population estimates are desired.

The use of the staining technique made it possible to obtain a quantitative estimate of the number of trout-perch between 46 and 103 mm in the lake. In terms of number per acre these figures range from 1186 to 1472 fish. Application of this method of marking small fish to other species present in Heming Lake should provide interesting statistics on population density and interaction between species.

ACKNOWLEDGMENTS

The authors wish to thank the summer students (1966) at Heming Lake who assisted so enthusiastically with these experiments.

The help of Dr L. Johnson, Mr P. D. M. Macdonald, and Mr R. T. Matsunaga, who reviewed the manuscript, is gratefully appreciated.

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The Occurrence and Retention of Dimethyl-β-Propiothetin in Some Filter-Feeding Organisms[1]

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ABSTRACT

Determinations of dimethyl-β-propiothetin (DMPT) levels are reported for 10 filter-feeding organisms, 1 browsing mollusc, 1 carnivorous mollusc, and 1 crustacean. Mature oysters, scallops, and the pteropod *Limacina helicina* showed high (ca. 0.1 mg/g) DMPT levels, mussels showed generally lower levels, and quahogs and a tunicate showed negligible amounts of DMPT. The periwinkle accumulated DMPT only when feeding on an alga rich in DMPT. Retention of DMPT over prolonged periods was studied in the oyster and in mussels.

Seasonal variations, year-to-year variations, and variations with locations were studied for oysters and to a limited extent for mussels. The findings are discussed in terms of food availability and feeding habits and indicate that together with tocopherol it may be possible to correlate DMPT levels with the availability of phytoplankters in the food intake or some species. It is suggested that phagocytes may be responsible for prolonged retention of DMPT in oysters.

INTRODUCTION

Molluscs have a tendency to selectively accumulate unusual organic materials from their environment. This is well-documented in the serious instance of paralytic shellfish poison (Medcof et al., 1947, 1966; Prakash, 1963, 1967; Bourne, 1965) which is associated with the natural occurrence of a particular phytoplankter food organism (*Gonyaulax tamarensis*). Polycyclic aromatic hydrocarbons are also of increasing concern in edible species from some areas (Perdriau, 1964; Mallet, 1961; Cahnmann and Kuratsune, 1957), and these probably are ingested when adsorbed on organic detritus and particulate matter which form an appreciable part of the food of estuarine species (Parsons, 1963; Jørgensen, 1966). Alternatively they could be absorbed from true solution in seawater (Jeffrey, 1966).

Some implications of the occurrence of dimethyl sulphide (DMS) and its precursor, dimethyl-β-propiothetin (DMPT), have been discussed elsewhere from the point of view of fishery products (Sipos and Ackman, 1964; Ackman, 1965; Ackman et al., 1965, 1966a, b, 1967; Lippa, 1965; Motohiro, 1962). These reports have not discussed in detail the occurrence of DMS and DMPT in shellfish and plankton feeders in general. Specifically, association of these materials with invertebrates has been limited to DMS and DMPT in the pteropod *Limacina helicina* (Motohiro, 1962; Ackman et al., 1965, 1966a, 1967; Lippa, 1965), DMS in the Pacific oyster *Crassostrea gigas* (Ronald and

[1]Received for publication September 14, 1967.

J. Fish. Res. Bd. Canada, 25(2): 267–284, 1968.
Printed in Canada.

Thompson, 1964), and associated acrylic acid in the Antarctic krill *Euphausia superba* (Sieburth, 1960). The present survey is intended to cover the extent and variation of assimilation of DMPT in a variety of filter-feeding animals (necessarily mostly sedentary molluscs) and some other types. Particular attention has been paid to fresh and captive oysters because of commercial implications in this valuable species.

EXPERIMENTAL

Samples

Most of the samples examined were collected near the Biological Substation of the Fisheries Research Board, Ellerslie, Prince Edward Island, and shipped the same day by air to Halifax, N.S. On receipt the samples were placed in tanks with running filtered seawater (Stewart and Power, 1963). Analyses were carried out as promptly as possible, usually in the period 24–36 hr after collection. Additional samples from P.E.I. and New Brunswick as designated in the Tables and Figures were similarly shipped by air. Oysters from Malagash, N.S., were purchased at a Halifax commercial outlet. Samples from Halifax Harbour bottom were collected in the course of cleaning seawater intake lines and related samples were collected below the low-tide zone of the adjacent shoreline. Periwinkles and horse mussels designated "laboratory" were found on growing *Ulva lactuca* collected near Antigonish, N.S., and transported with this growth to the laboratory in plastic bags filled with seawater. Terence Bay, N.S., samples were brought directly to the laboratory and analysed the same day. Shrimp from commercial fishing trials were iced between the time of catching and analysis. The sample of *Limacina helicina* was collected on *A. T. Cameron* cruise 105, July 29, 1965 (Baffin Bay, lat 70°90′40″N, long 60°42′30″W). The set (No. 205) was an oblique haul from 50 m to the surface. Identification of the species in the sample was based on recovery of 12 small shells from *L. helicina* and 1 large shell from *L. retroversa*. Most of the shells found were fragmented but the proportions indicated that the sample should be largely *L. helicina* (W. Templeman, private communication).

Holding Conditions

The tank seawater temperature reflected normal seasonal variations in temperature of the inwards bottom current in Halifax Harbour. In 1965 and 1966 the overall trends were: a gradual decline from 5 to 1 C in the period January–March; a steady rise from 1 to 10 C in the period March–August; a seasonal sharp rise to 15 C in September followed by a sharp decline to 6 C and a gradual rise from 6 to 9 C from early October to late November, followed by a steady decline through December. Water temperatures were manipulated only as tabulated and discussed for certain oysters.

Analyses

In early 1965 experiments, the samples (entire shellfish, including juice, or drained meat or parts) were blended for 2 min with distilled water (ratio

of from 1:2 to 1:6 as warranted by circumstances) in a 200-ml polyethylene container (Cat. No. 8485, Eberbach Corp., Ann Arbor, Mich.; attached to a standard Waring Blendor). From late 1965, homogenization was carried out with a Sorvall Omni-Mixer and 50-ml stainless steel cup assembly (Cat. OM-1047). The homogenates were filtered and a 7-ml aliquot of the filtrate was taken for analysis of DMPT content (Ackman and Dale, 1965). Analyses of most 1966–67 specimens were on a drained weight basis as detailed in the Tables and Figures. Very small periwinkles, horse mussels, and pieces of particular organs were occasionally minced by a scalpel blade with distilled water in the analysis vial in lieu of blending.

RESULTS AND DISCUSSION

Oysters

A typical analysis sequence for oysters is shown in Fig. 1. No particular attempts were made to determine free DMS in homogenates, and it may be assumed that a considerable part of any DMS present in the specimens would be removed by processing. At no time was any particularly strong odour of DMS noted on opening live shellfish. Although a mild DMS odour was noted on opening the dead scallops (Table I), and some clams, this was insignificant

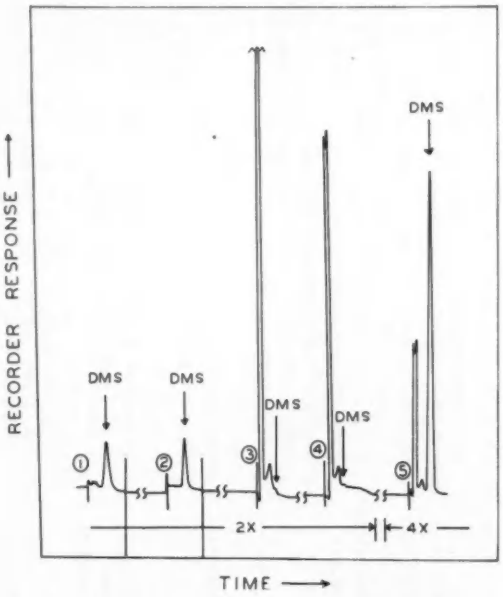

Fig. 1. Composite gas–liquid chromatogram of typical oyster analysis for DMPT. 1 and 2: DMS from standardization with known DMPT solution; 3 and 4: purges of head space over oyster blend and water; 5: DMS released from oyster blend on addition of alkali.

TABLE I. DMPT contents of some miscellaneous marine animals.

Species	Origin	Date Collected	Date Analysed	No. of specimens	Nature of sample[a]	Sample weight (g)	DMPT (*mg/g sample*)
Scallop (*Placopecten megallanicus*)	N.E. Pictou Island	Sept. 14, 1965	Sept. 25, 1965	4[b]	Adductor muscle	63	0.14
	" " "	"	"	4[b]	Adductor muscle	49	0.18
	" " "	"	"	4[b]	Visceral mass	124	0.17
	" " "	"	"	4[b]	Visceral mass	78	0.14
Common (blue) mussel (*Mytilus edulis*)	Halifax Hbr. bottom	Dec. 17, 1965	Dec. 17, 1965	5	Meat	7	0.005
	Halifax Hbr. bottom	"	"	5	"	5	0.007
	Halifax Hbr. shoreline	"	"	1	"	4.5	0.001
	Smelt Creek, P.E.I.	Aug. 17, 1965	Aug. 18, 1965	10	"	16.5	0.003
	England Bed, P.E.I.	"	"	2	"	8	0.001
	" " "	"	"	2	"	9	0.001
	" " "	"	"	2	"	8	0.004
	" " "	"	Nov. 19, 1965	2	Visceral mass	2.5	0.02
	" " "	"	Aug. 19, 1965	1	Meat	7	0.001
	" " "	"	Nov. 19, 1965	1	"	4	0.001
	Musquodoboit Hbr., N.S.	July 24, 1965	July 25, 1965	2	"	3	None
Ribbed mussel (*Volsella demissa*)	Smelt Creek, P.E.I.	Aug. 17, 1965	Aug. 18, 1965	10	"	16.5	0.01
	" " "	"	"	2	Visceral mass	1	0.02
	" " "	"	"	3	Meat	3	0.005
Horse (red) mussel (*Volsella modiolus*)	N.E. Pictou Island	Sept. 14, 1965	Sept. 16, 1965	1	Meat	28	0.001
	Laboratory (with *Ulva*)	Sept. 24, 1965	Sept. 25, 1965	4	Whole small animals	0.12	0.12
	" " "	Oct. 8, 1965	Oct. 9, 1965	1	Meat	0.95	0.04
	" " "	Oct. 8, 1965	Oct. 9, 1965	1	"	0.63	0.01
Soft-shell clam (*Mya arenaria*)	P.E.I. (Ellerslie)	Nov. 17, 1965	Nov. 18, 1965	1[b]	"	5	0.08
	" "	"	"	1	"	11	0.03
	" "	"	"	1	"	6.5	0.03

Bay quahog (*Venus mercenaria*)	P.E.I. (Ellerslie)	July 27, 1965	Aug. 6, 1965	3	Meat	57	0.001
	" "	"	Aug. 6, 1965	3	"	58	0.001
	" "	"	Nov. 26, 1965	1	"	6	None
	Paugh's Creek, P.E.I.	Aug. 17, 1965	Aug. 18, 1965	1	"	19	"
	Paugh's Creek, P.E.I.	Aug. 17, 1965	Aug. 18, 1965	1	"	31	"
	P.E.I. (Ellerslie)	Sept. 20, 1965	Sept. 22, 1965	3	"	35	"
Ocean quahog (*Arctica islandica*)	N.B. (St. Andrews)	Oct. 6, 1965	Oct. 7, 1965	1	"	15	"
	" " "	"	"	1	"	7	"
	" " "	"	"	1	"	11	"
	" " "	"	Nov. 29, 1965	1	"	7.5	"
Tunicate	Halifax Hbr. bottom	Dec. 15, 1965	Dec. 15, 1965	1	Whole animal	8	"
(Species unknown)	Halifax Hbr. bottom	Dec. 15, 1965	Dec. 15, 1965	1	Whole animal	6	"
Pteropod (*Limacina helicina*)	Baffin Bay	July 29, 1965	Sept. 19, 1965	?	Frozen whole animals	12	0.2–0.5
Periwinkle	Terence Bay, N.S.	Sept. 7, 1965	Sept. 7, 1965	12	Meat	7	None
(*Littorina littorea*)	Laboratory (with *Ulva*)	Sept. 24, 1965	Sept. 25, 1965	4	Whole small animals	0.25	2
Dogwinkle (*Thais lapillus*)	Terence Bay, N.S.	Sept. 7, 1965	Sept. 7, 1965	12	Meat	3	None
Shrimp	Nova Scotia waters	Aug. 28, 1965	Sept. 2, 1965	2[b]	Whole animals	6	0.01
(*Pandalus borealis*)	Nova Scotia waters	Sept, 10, 1965	Sept. 15, 1965	3[b]	Whole animals	4	None
Seawater (assoc. with *Ulva* etc.)	Nova Scotia waters, from laboratory	Oct. 8, 1965	Oct. 9, 1965	1[c]	See text	7(*ml*)	0.0003 (*mg/ml*)

[a]Meat implies whole animal body.
[b]Specimen arrived dead, held iced or frozen until analysis.
[c]Average of two separate analyses.

compared with that pervading the whole laboratory on opening a container of *L. helicina* packed in dry ice (Ackman et al., 1966a; cf. Ackman et al., 1967). Routine purges of the gas–liquid chromatographic head space (steps 3 and 4, Fig. 1) assured that little or no DMS remained to influence the determination of the DMS liberated from DMPT by alkali. Filtrates from homogenates of oysters gave results reproducible to ±5%. Unfiltered homogenates gave average results of about the same magnitude, but were generally only reproducible to ±20%, with occasional results much higher than normal, suggesting inclusion of particles or tissue very rich in DMPT.

A cursory investigation of the distribution of DMPT in different parts of oysters (Table II) showed that when DMPT levels were high the visceral mass contained several times the DMPT found in the rest of the animal. The stomach, "hepatopancreas," and kidney were very rich in DMPT. The reason for the high level in the adductor muscle was not clear, but this was a small portion and may have been contaminated with stomach contents. The difference between the inner and outer gills was of particular interest. This individual specimen (from a lot fished July 30, 1966) appeared to be typical of the lot since the total figure computed from the analysis of the parts was within the range of three control oysters from the same lot.

TABLE II. Investigations of the location of DMPT in specific parts of oysters.

Date				DMPT (*mg/g*)	
Fished	Analysed	Nature of sample	Weight (g)	In sample	For oyster
July 30, 1965	Aug. 2, 1965	Stomach, hepatopancreas, etc.	3.7	0.15	~0.06 (shell contents)
		Kidney	0.2	0.24	
		Inner gill	1.2	0.021	
		Outer gill	2.2	0.070	
		Adductor muscle	0.7	0.21	
		Juice	9	0.006	
July 30, 1965	Aug. 2, 1965	Control oyster	66	0.045	(Shell contents)
		" "	78	0.057	(Shell contents)
		" "	32	0.089	(Shell contents)
June 24, 1966	June 25, 1966	Visceral mass	0.5	0.016	0.004
		Balance of oyster	4	0.003	(Drained meat)
June 24, 1966	June 25, 1966	Visceral mass	0.5	0.032	0.008
		Balance of oyster	3	0.004	(Drained meat)
July 5, 1966	July 7, 1966	Visceral mass	0.9	0.063	0.054
		Balance of oyster	6.8	0.053	(Drained meat)
July 5, 1966	July 6, 1966	Visceral mass	1	0.23	0.10
		Balance of oyster	4	0.06	(Drained meat)
July 5, 1966	July 7, 1966	Visceral mass	0.8	0.75	0.20
		Balance of oyster	5.1	0.12	(Drained meat)
Aug. 23, 1966	Aug. 24, 1966	Visceral mass	1	0.020	0.018
		Balance of oyster	4.5	0.018	(Drained meat)

In two specimens examined in 1966 (fished July 5, 1966 and Aug. 23, 1966) there was no appreciable difference between the visceral mass and the balance of the oyster, although two others from the July 5, 1966 lot showed relatively higher figures for the viscera. The latter results were probably normal for oysters feeding on algae containing DMPT, but in the two unusual samples the overall levels were not particularly low. The observations of Bourne (1965) and Medcof et al. (1947) indicate that changes in accumulated paralytic shellfish toxin in gills and mantles of other species may lag behind changes in the level in the viscera as feeding conditions change.

Seasonal variations in DMPT levels for oysters are shown in Fig. 2–5. In 1965 the levels in four lots of oysters were around 0.15 mg/g for total shell contents (Fig. 2). These figures would be nearly twice as high if based on

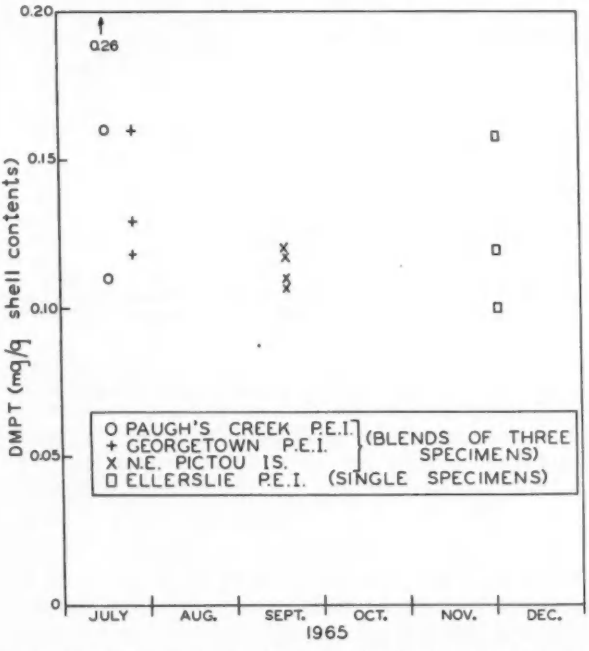

FIG. 2. DMPT contents of oysters (shell contents) taken at various locations in 1965.

drained meat, since the fluid shell contents contained relatively little DMPT. Drained meat was used for the 1966–67 figures. The 1966 samples from unspecified beds near Ellerslie (Fig. 3) were definitely lower in DMPT than the 1965 samples and appear to fit into the series for the Totten bed (Fig. 4) rather than the England bed (Fig. 5). There are some unusually high results in Fig. 2, 3, and 5, but the specified samples from the Totten and England beds confirm

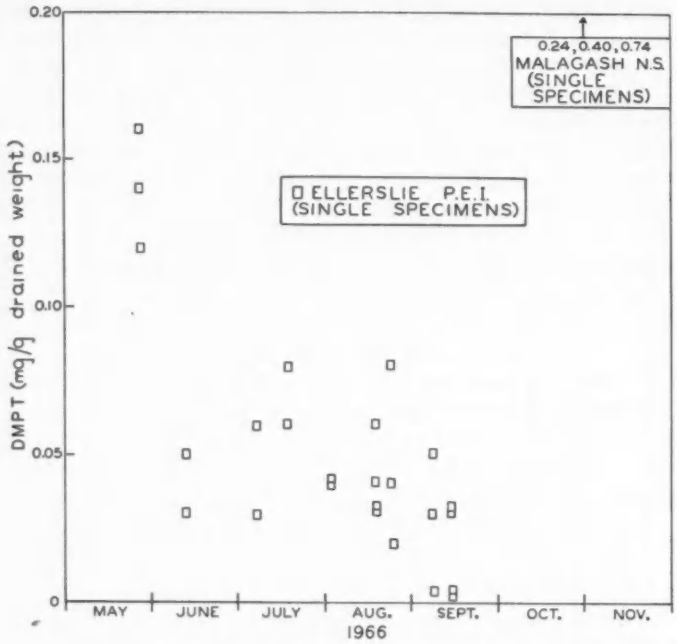

FIG. 3. DMPT contents of oysters (drained meat) taken from unspecified beds near Ellerslie, P.E.I., with one lot from Malagash, N.S., in 1966.

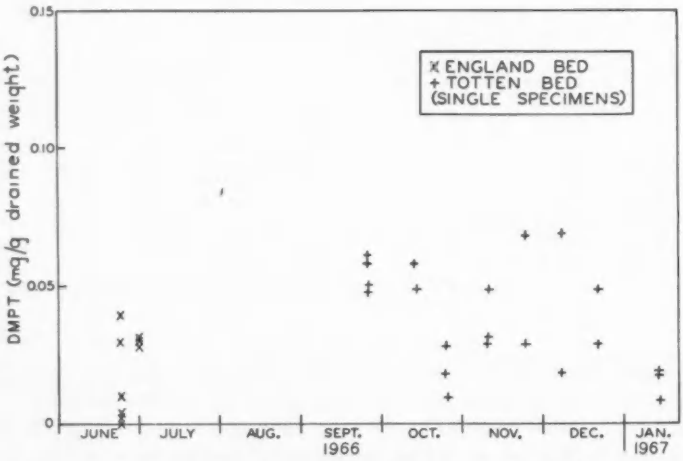

FIG. 4. DMPT contents of oysters (drained meat) taken from Totten bed, with two lots from England bed (near Ellerslie, P.E.I.) in 1966 and early 1967.

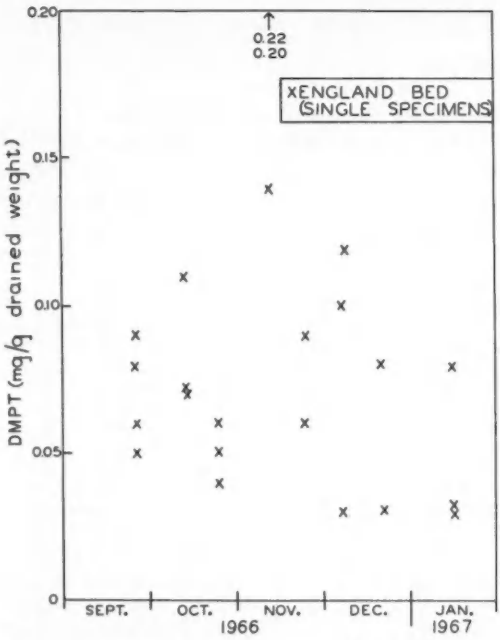

FIG. 5. DMPT contents of oysters (drained meat) taken from England bed (near Ellerslie, P.E.I.) in 1966 and early 1967.

that overall Ellerslie levels in 1966–67 were lower than in 1965. It is possible that a heavy infestation of gillworm, definitely more pronounced in the Totten bed (R. Drinnan, private communication), interfered with feeding, or these lower DMPT levels may reflect normal year-to-year fluctuations in water temperature and variations in number and types of unicellular marine algae containing DMPT.

Re-analysis of oysters after moderate periods of captivity (Table III) indicated that there was little or no decline in DMPT levels over periods of up to 2 or 3 months. The interpretation of these results must be considered in relation to water temperature. Oysters (specifically *Crassostrea virginica*) from the area under examination enter an anaerobic metabolic phase (von Brand, 1944; C. Medcof, private communication) below approximately 6 C. Haven and Morales-Alamo (1966) cite 2.8 C as a minimal temperature for feces production. The holding tank temperatures between January 1st and July 1st were normally below 6 C. After this, temperatures were above 6 C except for a brief period in late September (J. E. Stewart, private communication). The samples submitted to re-examination overlapped both temperatures above and below 6 C. There was no strong evidence of change in DMPT level associated with the type of metabolism in the animal.

TABLE III. Data for DMPT contents of oysters with re-analyses after captivity (>2 weeks) under normal tank conditions.

Date		No. of specimens	Nature of sample	Sample weight (*g*)	DMPT(*mg/g*)
Fished	Analysed				
Aug. 17, 1965	Aug. 25, 1965	3		66	0.11
	Aug. 30, 1965	3		70	0.26
	Sept. 3, 1965	3		30	0.16
	Oct. 22, 1965	1		10	0.12
Aug. 25, 1965	Aug. 25, 1965	2		37	0.12
	Aug. 26, 1965	2		27	0.13
	Sept. 3, 1965	2		30	0.16
	Nov. 22, 1965	1		9	0.12
	Jan. 12, 1966	1		5	0.11
Sept. 15, 1965	Sept. 17, 1965	2		25	0.11
	Sept. 17, 1965	2	Shell contents	23	0.11
	Sept. 22, 1965	2		20	0.12
	Sept. 22, 1965	2		18	0.12
	Nov. 24, 1965	1		11	0.14
	Nov. 24, 1965	1		13	0.15
	Jan. 12, 1966	1		5	0.10
Dec. 1, 1965	Dec. 2, 1965	1		11	0.10
	Dec. 2, 1965	1		5	0.12
	Dec. 2, 1965	1		10	0.16
	Jan. 13, 1966	1		12	0.14
	Jan. 13, 1966	1		5	0.15
June 7, 1966	June 8, 1966	1		18	0.03
	June 8, 1966	1		12	0.05
	Sept. 29, 1966	1		9	0.06
	Sept. 29, 1966	1		5	0.01
June 22, 1966	June 23, 1966	1		16	0.03
	June 23, 1966	1		12	0.01
	June 24, 1966	1		4	0.03
	June 24, 1966	1		3	0.04
	Sept. 30, 1966	1		5	0.06
	Sept. 30, 1966	1		5	0.09
June 30, 1966	July 7, 1966	1		5	0.3
	July 7, 1966	1		6	0.3
	Sept. 30, 1966	1		4	0.7
	Sept. 30, 1966	1		5	0.4
July 5, 1966	July 7, 1966	1	Drained meat	8	0.06
	July 7, 1966	1		6	0.03
	Sept. 27, 1966	1		7	0.06
	Sept. 27, 1966	1		10	0.07
July 19, 1966	July 19, 1966	1		5	0.08
	July 19, 1966	1		10	0.06
	Sept. 27, 1966	1		7	0.08
	Sept. 27, 1966	1		6	0.05
Aug. 2, 1966	Aug. 3, 1966	1		6	0.04
	Aug. 3, 1966	1		10	0.04
	Oct. 3, 1966	1		7	0.02
	Oct. 3, 1966	1		7	0.06
Aug. 15, 1966	Aug. 19, 1966	1		7	0.04
	Aug. 19, 1966	1		6	0.03
	Aug. 19, 1966	1		7	0.06
	Aug. 19, 1966	1		8	0.03
	Sept. 30, 1966	1		7	0.05
	Sept. 30, 1966	1		4	0.04

Over longer periods (up to 4 months), as shown by the samples in Table IV (lot fished May 25, 1966), there was probably some decline in DMPT. This period would include most of the period of water temperature above 6 C and the oysters should have been open and metabolically active under aerobic conditions, but with little or no food intake. After a further period of 4 months, mostly in the anaerobic condition, a definite decline in DMPT was observed. The shock of an alteration in water temperature from approximately 1 to 12 C over a period of 48 hr was fatal to some of these oysters (fished May 25, 1966) which were evidently in a weakened condition. All of

TABLE IV. Effect of prolonged captivity and thermal shock treatment on DMPT content of oysters from Ellerslie, P.E.I. Data for individual oysters (drained meat).

Dates	Action taken	Sample weights (g)	DMPT (*mg/g*)
May 25, 1966	Fished		
May 27, 1966	Analysed (from normal tank)	13, 18, 11	0.16, 0.14, 0.12
Sept. 29, 1966	Analysed (from normal tank)	8	0.03
Mar. 1, 1967	Raised tank temperature to 15 C		
Mar. 5, 1967	Analysed (live specimens, 15 C)	5, 5, 10, 8	0.02, 0.008, 0.006, 0.008
Mar. 5, 1967	Analysed (dead specimens, 15 C)	8, 8	0.01, 0.03
Mar. 5–7, 1967	Tank temperature accidentally raised to 50 C		
Mar. 7, 1967	Analysed (dead specimens, 50 C)	7, 8, 3	0.002, 0.05, 0.001
Jan. 14, 1967	Fished		
Jan. 19, 1967	Analysed (from normal tank)	12, 11, 8	0.08, 0.03, 0.03
Mar. 5, 1967	Analysed (from normal tank)	5, 5, 6	0.02, 0.03, 0.02
Mar. 1, 1967	Transferred to tank at 15 C		
Mar. 5, 1967	Analysed (live specimens, 15 C)	8, 12	0.02, 0.04

the different lot (fished Jan. 14, 1967) survived the same transition. Inadvertently the temperature escalated to 50 C over a weekend, resulting in the death of all of the remaining oysters. In view of the known thermal instability of DMPT (Motohiro, 1962; Ackman and Dale, 1965; Ackman et al., 1966a), it was interesting to find that there still remained substantial DMPT residues in the latter animals. These observations of the stability of DMPT in oyster tissue confirm that there is no active system liberating DMS from DMPT. Hence, DMS observed in live oysters and in other live shellfish must arise principally from the breakdown of DMPT by enzymes naturally present in the algae and activated by the death of the algae (cf. Challenger, 1959).

The digestion of food by the oyster *C. virginica* has been reviewed by Galtsoff (1964) and the digestive system studied in detail by Shaw and Battle (1957). A comparable study of the anatomy and function of the stomach in the Japanese pearl oyster, *Pinctada martensii*, has been carried out by Kuwatani (1965). In general, food and other particles are sorted efficiently at the stomach

entrance and if unsuitable for digestion are rejected. Most suitable materials, including some unicellular algae, pass through the digestive system in a few hours (Galtsoff, 1964). Gross storage of food particles is apparently only temporary (Jørgensen, 1966; Kuwatani, 1965). Intact algal cells are often seen in the digestive tract and the so-called "hepatopancreas" area as well as in feces. There is no evidence that the diverticulated organs retain materials for any appreciable time, ciliary action being quite pronounced. On the other hand algal cells are reportedly stored in surrounding tissue (Galtsoff, 1964). Phagocytes (amoebocytes) are very numerous in the digestive tissues and actively ingest algae. Phagocytes are also reported as abundant on the gill surfaces (Galtsoff, 1964), an interesting observation since the gill surfaces normally expel unwanted particles and rapidly pass more suitable materials to the digestive tract proper (Jørgensen, 1966).

In higher animals such as salmon (Motohiro, 1962) and cod (Ackman et al., 1966a) DMPT presumably accumulates in the free form in flesh and liver, possibly in the intercellular fluids, and is rapidly excreted when intake diminishes. The retention of DMPT in the oyster is so rigid (Tables II–IV) that it is unlikely that DMPT is present in a free form. The status of DMPT in unicellular algae is not known although there are indications (Ackman et al., 1966b) that in some species at least it is bound in structural components as reported for multicellular algae (Nicolai and Preston, 1953).

The following hypothesis is advanced to explain the apparent retention of DMPT by oysters in particular, and possibly by some other filter feeders. An intact algal cell is ingested by a phagocyte. This results in the death of the algal cell and immediate spontaneous release of a small amount of DMPT or, more likely, the normal DMPT breakdown product, acrylic acid. The latter is a powerful antimetabolite (Sieburth, 1960, 1961, 1964), and might well prevent further catabolism of the algal cell by the phagocyte. The existing enzymes in the phagocyte would concurrently suppress further breakdown of DMPT by the enzymes naturally present in the algal cell. Since the phagocyte would be intact although metabolically inactive, the oyster would presumably take no action in respect to it, and the acid conditions prevalent in the oyster digestive system (Galtsoff, 1964) would favour stabilization of the DMPT. Among other points, this hypothesis conveniently accounts for the presence of DMPT in the gill areas of oysters which had been held for 24 hr in organism-free seawater warm enough to ensure that they would be open and metabolically active. The action of the gill surfaces would normally remove any mechanically adherent algal cells. The accumulation of an algal toxin in the hepatopancreas of California sea mussels *Mytilus californianus* (Schantz et al., 1966), and the relationship between amount of toxin and number of algal cells ingested by molluscs (Prakash, 1963, 1967) suggests a similar mode of accumulation for algal toxin. The more complex algal toxins might be selectively bound by interaction with shellfish protein; this seems less likely with the simpler DMPT molecules.

Other Species

Unfortunately, the scallop specimens examined were dead on arrival (Table I) but proved to be unexpectedly rich in DMPT; further research is indicated for this species. Scallops fished from the Virginia banks have a distinctly different flavour (F. C. Read, personal communication) from those fished in areas closer to Nova Scotia such as George's Bank. This might be related to environmental factors such as DMPT levels in phytoplankton.

The results for the Halifax Harbour common mussel samples parallel the analyses obtained for tocopherol levels in samples from the same locations (Ackman and Cormier, 1967). Similar results were observed in certain oysters (see below). Both DMPT and tocopherol, associated specifically with phytoplankters, were much lower in the shoreline samples than in the bottom samples. The shoreline mussels therefore must ingest detritus as a much higher proportion of food than the bottom mussels. The mussels fished from the England Bed (July 17, 1965) had relatively low DMPT levels but DMPT was still detectable after 3 months of captivity. DMPT accumulation in the visceral mass was evident. The Musquodoboit Harbour sample came from a brackish environment in which purely marine life forms are scarce. Possibly the phytoplankters available were types lacking DMPT (Ishida and Kadota, 1967).

The ribbed mussels had relatively high DMPT levels; their visceral masses were somewhat enriched in DMPT. Several months of captivity did not reduce DMPT levels appreciably.

The large horse mussel was obtained in fishing for scallops on the N.E. Pictou Island bed, but had very little DMPT in comparison with the scallops. The small horse mussels identified as "laboratory" were firmly attached to growing *Ulva lactuca* collected for DMPT analysis (Tocher et al., 1966). It is probable that the high DMPT levels in the mussels merely reflect favourable conditions for a heavy growth of phytoplankters rather than an association with *Ulva*, although sporulation of *Ulva* cannot be ruled out as a contributing factor. The difference between the very small animals (for which shells were included in the sample weight) and the other samples is not excessive when compared with results for the oyster.

The soft shell clam showed modest DMPT levels. DMS has been reported to develop in stored irradiated meat of this species (Ronsivalli and Slavin, 1965; cf. Ronald and Thompson, 1964), and dilute DMS solutions were considered by taste panels to be typical of odours associated with clams (J. M. Mendelsohn and R. O. Brooke, private communication).

The quahogs are of particular interest since many persons regard the bay quahog as having a "strong" flavour in comparison with oysters and soft shell clams, whereas ocean quahogs are not handled commercially since they are reported on occasion to have objectionable odours and tastes when cooked (Chandler, 1965). Although materials chemically related to DMPT are progenitors of strong flavours in many vegetables (see Ackman et al., 1966a; Challenger, 1959), there is no evidence that DMPT accumulates in either

quahog in any significant quantity. Various quahogs are apparently efficient filter feeders (Jørgensen, 1966) and the presence of tocopherol in quahogs in reasonable amounts (Ackman and Cormier, 1967) suggests that phytoplankters must form an appreciable part of the food intake. A basic difference in the food assimilation process in the quahog as compared with oysters and some other filter feeders could account for the differences in DMPT levels. Preliminary filtration may exclude certain phytoplankters on a size basis in one species and not in another (see below).

The tunicate from the bottom of Halifax Harbour also showed no DMPT accumulation although mussels collected at the same time showed modest levels. A basic difference in food assimilation is again indicated.

The pteropod *Limicina helicina*, which stimulated this research program, differs in mode of feeding from most of the other species examined (Jørgensen, 1966). Gills are absent, and collection takes place on mucus-covered surfaces. The mucus is then transferred in strings to the mouth. The details of the digestive system available fail to account for any storage of DMPT but phagocytosis is important in some gastropods (Owen, 1966a). The restricted distribution of high concentrations of this species and its association with particular water masses (Ackman et al., 1967; Dunbar, 1942; Kerswill, 1940; Kielhorn, 1952) suggests that dense populations are probably associated with blooms of particular phytoplankters rich in DMPT. Unfortunately it has not been possible to identify any particular unicellular algae in collections of *L. helicinia* or *L. retroversa* (W. Templeman, F. H. Grainger, private communications).

The common periwinkle is moderately mobile, but may browse for extended periods on particular sessile algae. The complete absence of DMPT in the Terence Bay sample was not unexpected, as DMPT has not been associated with more than a few species of sessile algae (Challenger, 1959). Conversely, the high level in the animals browsing on *Ulva lactuca*, known to be rich in DMPT, was a reasonable finding. The water in which the *Ulva* had been held for 24 hr showed some DMPT, but it is not known if this is normally released by the intact plant or whether it accumulated from damaged parts (Ackman et al., 1966b; Challenger, 1959).

Two carnivores were included in this survey. The dogwinkle associated with the Terence Bay periwinkles showed no DMPT, and it is possible that even if feeding on animals containing DMPT, the mechanics of feeding would reduce transfer and retention of DMPT. High concentrations of paralytic shellfish poison are known to occur at times in digestive glands of the rough whelk (*Buccinum undatum*) (Medcof et al., 1966). It is perhaps significant that in this carnivorous species paralytic shellfish poison levels drop rapidly when intake ceases (Caddy and Chandler, 1967). One of two lots of shrimp showed a fairly high DMPT content, but it is not possible to state whether this arose from primary ingestion of phytoplankters or secondary ingestion along with herbivorous animals. The postmortem stability of DMPT in the shrimp is of interest (see Ackman et al., 1966a, 1967).

GENERAL COMMENTS AND POSSIBLE VALUE OF DMPT IN RESEARCH ON FILTER FEEDERS

Although the specific association of DMS with undesirable odours in commercial fishery products has been limited to chum salmon (*Oncorhynchus keta*) (Motohiro, 1962), rock sole (*Lepidopsetta bilineata*), and Pacific cod (*Gadus macrocephalus*) (Lippa, 1965) in the Pacific; and cod (*Gadus morhua*) (Sipos and Ackman, 1964; Ackman et al., 1967) in the Atlantic, there are other reports which bear on this matter. "Stinkers" is probably an apt name for commercially handled fish such as haddock (*Melanogrammus aeglefinus*) which may be a problem in the Lofoten area and elsewhere in the summer (Devik, 1956; Smith, 1956). Brisling sardines (young *Clupea sprattus*, 9–12 cm in length) often eat *Limacina retroversa* (Norwegian "Kruttåte Spiralis") or *Limacina helicina* (Norwegian "Flueåte") and for this reason the brisling are held in the nets for a period to clear themselves of gut contents (Anon, 1965; Kr. Fr. Wiborg, personal communication).

These reports involve the occurrence in water from higher latitudes of the pteropods *L. helicina* and *L. retroversa*. Notwithstanding this apparent specific association with Arctic water masses, there is good reason to believe that a substantial proportion of phytoplankters available to filter feeders in temperate latitudes contain DMPT. In the original examination of marine unicellular algae, DMPT was found in four out of seven classes (Ackman et al., 1966b). Additionally, DMPT has been confirmed as a basic constituent of a new class of unicellular algae, the *Prasinophyceae*, as well as occurring in some *Chlorophyceae* (Craigie et al., 1966, 1967). Specific information on phytoplankters in the area of the Ellerslie oyster beds is lacking, but at least one species common in the spring plankton bloom in the western North Atlantic, *Skeletonema costatum*, is known to contain DMPT.

The specific conditions under which particular shellfish can flourish may occur in very restricted areas with sharp differentiation of populations over apparently similar bottom areas (see Galtstoff, 1964, for oysters). The samples from specified beds (England and Totten) in the Ellerslie area of P.E.I. obtained at approximately 2-week intervals in the latter half of 1966 caused concern since various shipments ostensibly from particular beds were physically quite different in appearance. Shape, adherent mud colour, damage from sponges, etc. were characteristics which were usually homogeneous in one lot, but often different from lot to lot. Most of these lots, however, came from the immediate vicinity of fixed markers and thus from within an area of only a few hundred square yards (R. Drinnan, private communication). Within this limited area proportions of various types of organic suspended matter may have varied enough to affect DMPT levels somewhat. There is evidence, however, that for a given species, DMPT levels correlate roughly with the intake of phytoplankters; this could provide useful comparative data on phytoplankter availability. A comparison of tocopherol and DMPT levels in Halifax Harbour mussels (see above) agreed with a probable local distinction in food type. This point was further investigated with oysters

collected in October 1966. DMPT levels (average of three individuals) were 0.025 mg/g and 0.050 mg/g respectively for the Totten and England beds, and 0.25 mg/g for Malagash oysters. Tocopherol levels in two different lots of Totten bed oysters (average of three) from the same date were respectively 0.19 and 0.58 μg/g, whereas in three Malagash oysters examined the average level was 1.02 μg/g. Tocopherol levels drop rapidly in captivity (Ackman and Cormier, 1967); the original levels in the commercially handled Malagash samples may well have been appreciably higher. By contrast, DMPT levels should be relatively unaffected. The physiological status of these specimens was not recorded but there is a rough correlation for the two components known to be of phytoplankter origin.

An interspecies comparison of DMPT levels is of less definite value. In addition to possible differences in food assimilation in the digestive tract the preliminary filtering may differentiate between algal cells by size. For example it is reported that oysters may accept algal cells in the size range 2–3 μ that quahogs would reject (Owen, 1966b). This is a controversial area (Galtsoff, 1964; Jørgensen, 1966) but DMPT analyses in laboratory feeding experiments might well supplement conventional techniques used in studying feeding of molluscs and other filter feeders.

ACKNOWLEDGMENTS

The authors wish to express their appreciation of the considerable efforts of R. E. Drinnan and associates of the Fisheries Researd Board of Canada, Biological Substation, Ellerslie, P.E.I., in collecting and shipping samples. The advice and assistance of W. Templeman, FRBC, St. John's Nfld. Laboratory, in collecting *L. helicina*, and of Mrs R. Lord and other scientists of the FRBC, Biological Station, St. Andrews, N.B., is also acknowledged.

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Feeding Biology of the Black Crappie, *Pomoxis nigromaculatus*[1]

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ABSTRACT

In Lake Opinicon, Ontario, the diet of the black crappie, *Pomoxis nigromaculatus*, undergoes a progressive change from one in which planktonic Crustacea and small-bodied Diptera larvae predominate (in fish of body length from about 60 to 115 mm), to a diet of insect larvae and fish (in fish 160–240 mm). Most food items prove to be midwater forms and the Diptera larvae are almost entirely *Chaoborus* and *Procladius*, which are free-swimming in the water column at night.

An unusual feature is the prolonged nature of the Cladocera-Copepoda eating phase, which continues into year III and to a body length of about 160 mm. Gill-raker counts show *P. nigromaculatus* to have a specialized screen with 25–29 rakers on the first arch. In this it differs from cohabiting centrarchids in Lake Opinicon, *Ambloplites rupestris*, *Micropterus salmoides*, and *Lepomis macrochirus*, in which the rakers on the first arch number only 8–12. In these species plankton feeding is restricted to the earlier stages.

INTRODUCTION

Notwithstanding that dietary studies have been carried out on Great Lakes fishes for over 50 years, the food niches of relatively few have been properly delineated. This is a prerequisite to an understanding of interspecific relations, proper management, and future studies in secondary productivity.

In the present paper the food of the black crappie (*Pomoxis nigromaculatus*, Centrarchidae) is investigated. The work is based on a study of the population of Lake Opinicon, Ontario, and a consideration of literature on food in other parts of the range. The gill-raker formulae of the species and three other centrarchids are discussed in relation to plankton feeding.

METHODS

Lake Opinicon is a small lake in the Rideau system with dimensions of 6 × 1 miles, a surface area of 2200 acres, and a maximum depth of 35 ft. It is usually ice-covered from late December to early April.

The collections were made in the latter half of each month between the years 1962 and 1965, and were kept strictly comparable. Most fish were obtained by a 200-yard gill net extending from the foreshore out towards the middle of the lake. The main sites were near the Queen's University boathouse, in water ranging from 6 to 20 ft in depth. Accessory netting was carried out by seine (by swimming with the net and from a boat) along areas of sloping

[1]Received for publication March 6, 1967.

J. Fish. Res. Bd. Canada, 25(2): 285–297, 1968.
Printed in Canada.

shoreline, where the species regularly occurred. The whole 24-hr period was covered on each occasion to span the different feeding periods. The stomachs analysed here represent samples of the collections.

The procedure followed in removing and analysing the stomach contents was as set out in Keast (1965). Data are expressed as percentage volumes of each item for the entire series, and as numerical counts averaged for 10 fish. A brief account of the food of 75–115 mm *P. nigromaculatus* in the shallows of Birch Bay, Lake Opinicon, has previously been published as part of a study of resource subdivision amongst the cohabiting species in that bay (Keast, 1965). In that work feeding was interpreted relative to seasonal fluctuations in numbers of the prey organisms.

THE FOOD IN LAKE OPINICON, ONTARIO

It is obvious from the Lake Opinicon study that *P. nigromaculatus* occupies a clearly defined food niche. The diet (Table I) is made up largely of three major types of food: planktonic Crustacea, Diptera larvae and pupae, and fish. Ephemeroptera and Zygoptera nymphs are prominent in 2 or 3 months and Amphipoda (*Hyalella*) in 1. Limited quantities of winged Diptera (especially chironomids) are picked up from the surface.

Seasonal differences in the diets of the three size-groups may be summarized as follows:

60–115 mm fish: In May, Diptera larvae and pupae (48% by volume), Ephemeroptera numphs (16%), and Cladocera and Ostracoda (each 13%) predominated. In June and July, Diptera larvae and pupae accounted for 62% and 73% of the volumes, respectively, remaining the major foods. Fish fry made up 19% of the volume in the former month and surface insects 11% in July. Diptera larvae and pupae represented 49% of the volume in August but only 38% in September. Cladocera and Copepoda, which reach numerical peaks in many parts of the lake in late summer (McBurney, MS, 1951; Keast, 1965), made up 25% of the volume in August and 42% in September.

116–160 mm fish: Diptera larvae and pupae were the major items of diet in all months but September. In May, Ephemeroptera nymphs made up 26%, and Anisoptera nymphs 28%, of the stomach volumes, largely replacing small Crustacea in the diet. In July, fish made up 40% of the volume, Diptera larvae being only 47%. Amphipoda accounted for 34% of the volume in September. Small planktonic Crustacea composed 21% and 23% of the volume, respectively, in August and September.

161–240 mm fish: Planktonic Crustacea were virtually absent from the diet. Diptera larvae and pupae accounted for volumes of 61%, 47%, 32%, 53%, and 40% in the 5 months. The increasing importance of fish is seen from them making up volumes of 17%, 39%, 65%, 42%, and 11% of the volume respectively. Ephemeroptera nymphs accounted for 27% of the volume in September.

In numbers of individuals of these various food items Cladocera were sometimes very prominent: 2241 per 10 stomachs in August and 830 in Sep-

TABLE I. Food of *Pomoxis nigromaculatus*, Lake Opinicon. Numbers of food organisms are averaged for 10 fish. Per cent volumes refer to entire series. Numbers of fish in the samples are given below the names of the months.

	Body length, 60–115 mm									
	May 33		June 39		July 39		August 40		September 38	
Food organisms	No.	% Vol.	No.	% Vol.	No.	% Vol.	No.	% Vol.	No.	% Vol.
Cladocera	186	13	68	2	112	5	2241	16	830	30
Copepoda	48	3	2	1	35	3	327	9	190	12
Ostracoda	156	13	6	1	10	1	4	1	50	11
Amphipoda	5	3	1	–	4	1	1	1	15	4
Ephemeroptera N[a]	18	16	3	1	2	1	3	5	4	2
Zygoptera N.	1	–	2	5	1	2				
Trichoptera L.[a]	2	2			1	–	2	1		
Chironomid L.	24	18	19	14	38	24	4	1	15	13
Chaoborid L.	16	10	21	12	24	11	83	24	20	22
Diptera pupae	45	20	94	36	53	38	32	24	3	3
Diptera and Hymenoptera adults			10	6	26	11	8	5		
Plecoptera									1	1
Hydracarina	1	–	10	1	1	–	3	1		
Fish			2	19	1	3	1	11		

	Body length, 116–160 mm									
	May 27		June 35		July 29		August 33		September 25	
Food organisms	No.	% Vol.	No.	% Vol.	No.	% Vol.	No.	% Vol.	No.	% Vol.
Cladocera	84	2	42	1	41	5	426	17	899	20
Copepoda	7	1			1	–	18	4	103	3
Ostracoda	7	1			1	–	3	1		
Amphipoda	2	–	15	1					20	34
Ephemeroptera N.	31	26	6	2	2	2	1	1		
Zygoptera N.			6	6						
Anisoptera N.	11	28								
Trichoptera L.			3	–	1	–	1	1	3	1
Chironomid L.	209	14	240	18	8	4	8	8		
Chaoborid L.	92	9	134	34	99	4	50	16	45	16
Diptera pupae	34	17	113	12	173	39	77	41		
Diptera and Hymenoptera adults	9	1	9	2	7	3	9	7	7	3
Hydracarina	8	1	12	1	8	2	2	1		
Fish			4	23	7	40	1	3	4	23

[a]N, nymphs; L, larvae.

(*Continued*)

TABLE I. Food of *Pomoxis nigromaculatus*, Lake Opinicon. Numbers of food organisms are averaged for 10 fish. Per cent volumes refer to entire series. Numbers of fish in the samples are given below the names of the months. — (*Concluded*)

	Body length, 161–240 mm									
	May 27		June 27		July 29		August 25		September 23	
Food organisms	No.	% Vol.	No.	% Vol.	No.	% Vol.	No.	% Vol.	No.	% Vol.
Cladocera	35	1	65	–	30	–	62	2	22	2
Copepoda	1	–			1	–	1	–		
Amphipoda	1	1	1	–			1	–	112	7
Ephemeroptera N.	14	6	20	6	1	1	1	–	57	27
Zygoptera N.	21	10	15	7	1	1				
Anisoptera N.	3	3								
Trichoptera	2	–	2	1					29	7
Chironomid L.	61	10	165	16	6	2	4	2	6	1
Chaoborid L.	370	39	75	6	42	8	50	23	76	38
Diptera pupae	24	12	280	25	19	22	47	28	8	1
Diptera and Hymenoptera adults	15	1					6	2		
Coleoptera							1 adult	1	10 larvae	5
Hydracarina	1	–	2	–			1	–		
Fish	1	17	4	39	9	65	6	42	2	11

tember in the 60–115 mm group, and 426 and 899, respectively, in the 116–160 mm group. By contrast, the maximum number in the fish of over 160 mm in length was only 62 per 10 stomachs; hence it is obvious that Cladocera were no longer sought and/or that the gill raker screen was less effective. Counts of Copepoda were 327 and 190 per 10 stomachs in August and September, respectively, in the smallest size-group. This group contained Ostracoda at the rate of 156 per 10 stomachs in May.

The numbers of *Chaoborus* larvae ranged from 16 to 83 per 10 stomachs in the smallest group, 34 to 134 in the intermediate group, and 42 to 370 in the largest fish. Equivalent figures for chironomid larvae are 4–38, 8–240, and 4–165. For Diptera pupae they were 3–94, nil–113, and 8–280.

The highest counts for Ephemeroptera nymphs were 31 in May and 57 in September, in the medium-sized and large groups, respectively. The numbers of Zygoptera nymphs were uniformly small, and the maximum figure for Diptera (mostly chironomid) adults was 26 per 10 stomachs.

The highest figure reached for fish was nine per 10 stomachs, but seldom did one individual contain more than two.

The genera of Cladocera represented were as follows: July — *Chydorus*, *Daphnia*, *Bosmina*, plus a few *Sida* and *Diaphanosoma*; August — *Chydorus*, *Bosmina* (small-bodied), plus a few larger *Bosmina*; September — *Chydorus*,

Bosmina and *Alonella*. *Cyclops* predominated among the copepods. In the plankton, small-bodied species predominated, and inshore species were most abundant.

The chironomid larvae and pupae were kindly identified by Dr A. L. Hamilton, Canada Department of Agriculture, Ottawa, Ont. The pupae reached their greatest diversity by far in May, the following genera and subfamilies being represented: *Procladius* (most common), *Harnischia* (subgenus *Cryptotendipes*), *Ablabesmyia*, *Endochironomus*, *Chironomus* (*Dicrotendipes*), *Tribelos*, *Chironomus* (large-bodied), *Cryptochironomus*, plus unidentifiable members of the Tanytarsinae. Most of those in June were *Chironomus* (*Dicrotendipes*) and members of the Pentaneurini, plus a few *Procladius* and *Harnischia*. In July they were *Harnischia*, *Chironomus*, and many Tanytarsinae. In August they were mostly *Procladius* and *Cryptochironomus*. In September, *Chironomus* (*Dicrotendipes*), *Procladius*, and *Harnischia* predominated, and some Tanytarsinae were also present.

Amongst the larvae, *Procladius* predominated in May and June, one series of six stomachs containing no fewer than 300 individuals in the latter month. *Polypedilum* and *Chironomus* (*Dicrotendipes*) were also represented, plus some free-swimming ceratopogonids (*Culicoides*). The July larvae were *Procladius*, *Endochironomus*, and Pentaneurini, and the August ones were *Procladius*, *Harnischia* (one), *Cryptochironomus*, Pentaneurini, and *Chironomus* sensu strictu. In the September series the larvae were *Procladius* and *Chironomus*.

The fish species taken by *P. nigromaculatus* included *Perca flavescens*, *Lepomis macrochirus* and *L. gibbosus*, an occasional small member of its own species, *Notropis heterodon*, *Notemigonus crysoleucas*, *Pimephales notatus*, *Micropterus salmoides*, and *M. dolomieu*. Of those that could be identified, between one-third and one-half were yellow perch, this being the species of which the young members share the open water habitat of *P. nigromaculatus*. The young of most of the other species tend to be more in water 6 ft deep or less.

Amongst the minnows, *Notropis heterodon* and *Notemigonus crysoleucas* are commonly found in the weedy areas, and the former, a small species, ranked third in numbers after perch and sunfish.

The fish prey of *P. nigromaculatus* were characterized by their small size. Apart from fry and fingerlings most of the perch eaten ranged from 35 to 60 mm in total length, and the biggest sunfish, found in a 240-mm crappie, was only 68 mm in length.

The nature of foods consumed by *P. nigromaculatus* indicates that it is to a large extent a midwater feeder, and that there is a nocturnal component in its activities. The masses of free-swimming *Chaoborus* larvae and pupae, and planktonic Crustacea, move upwards through the water column after dark, becoming accessible. Chironomid adults emerge largely during darkness. Though many chironomids emerge immediately the pupae reach the surface, this emergence may be delayed for several hours in *Procladius* (Mundie,

1959). These are thus more susceptible to predation by *Pomoxis* than other forms, and this explains the greater frequency of their occurrence in stomachs.

Amongst larval chironomids eaten, those of *Procladius* were the most common found. Both *Procladius* and *Ablabesmyia* are predaceous and free-swimming. A number of the other genera are, however, tube-dwellers. *Pomoxis*, with its dorsally directed mouth (see Keast and Webb, 1966) and fine gill rakers (see later), subject to fouling by mud, certainly could not obtain these larvae from beneath the substratum (as can, for example, *Ictalurus nebulosus*). It is now, however, becoming appreciated that chironomid larvae of many genera emerge from their tubes and wander over the bottom after dark (A. L. Hamilton, personal communication). Still more surprising are the findings of Mundie (1959) that certain chironomid larvae may be found at the surface at night, along with *Hyalella* (Amphipoda) and various Hydracarina. It is presumably during this migration that tube-dwelling species are obtained by *Pomoxis*.

Childers and Shoemaker (1953) have demonstrated, by means of feeding studies in aquaria, that in Wisconsin *P. nigromaculatus* is partly a nocturnal feeder. Of the 407 small minnows used as food 56% were consumed at night (6 PM–6 AM), 30% at dawn (6–8 AM), 11% at dusk (4–6 PM) and only 2% by day (8 AM–4 PM). Pearse (1919) noted that most *Pomoxis* stomachs are empty in the afternoons, full at night.

An attempt was made (Table II) to determine the feeding chronology of *P. nigromaculatus* in Lake Opinicon by netting series of fish at 2-hr intervals throughout the 24-hr period, and determining the weight of the stomach contents for each individual and series. Fish between 60 and 140 mm body length were used and those collected in June, July, and August are grouped. Results are expressed as a ratio of the body weight, to equate fish of different sizes.

TABLE II. Feeding periodicity in *P. nigromaculatus* based on weight of stomach contents per gram of body weight for different times of day, June to August, 1965–66.

Time netted	No. of fish	Weight of food in stomach per gram of body weight (g)
0500	34	0.0045
0700	26	0.008
0900	34	0.010
1100	48	0.005
1300	12	0.012
1500	19	0.005
1700	18	0.0009
1900	18	0.004
2100	38	0.0018
2200	10	0.001
2400	19	0.001
0300	29	0.010

Table II shows that stomachs were fullest between 7 and 9 AM, at about 1 PM, and at 3 AM. This would indicate feeding periods in the early morning (from about 5 to 8 AM), in late morning, and between midnight and 2 AM. A late morning feeding period could, however, be illusory, as the size of the sample was small.

A feature of the stomach content weights was the great individual variation, and for this reason standard deviations are not given. This variation applied even at times of maximum average stomach fullness and irrespective of the month. Thus, one series of 14 fish collected at 7 AM in July 1967 had average stomach content weights of 0.0006 g per gram body weight, another of 12 had 0.042. During this same month mean 3 AM stomach weights of two groups of fish were respectively 0.001 and 0.022. These findings stress the necessity of only considering large series of fish. Only a small minority of individuals in the total series had empty stomachs, indicating that feeding usually recommences before the stomach empties completely.

The feeding times indicated for Lake Opinicon agree broadly with those determined by Childers and Shoemaker (1953) for Wisconsin.

THE FOOD IN OTHER PARTS OF THE RANGE

Food studies on *P. nigromaculatus* have been carried out in Wisconsin (Pearse, 1919), Illinois (Forbes and Richardson, 1920), Iowa (Bailey and Harrison, 1945), Tennessee (Dendy, 1946), Florida (Reid, 1949), and Texas (Mitchell, MS, 1945). Although the sampling procedures, methods of analysis, and numbers of fish used by the older workers are inadequate by modern standards the studies do bring out clearly that the diet is composed of the same basic organisms throughout the species range. The proportions of the major items differ. Agreement is closest in the northern parts of the range (Ontario, Wisconsin, Illinois), but the body sizes at which the major dietary shifts occur may differ between the northern and southern parts and according to area in the south. The southern populations contrast with the northern ones also in that feeding is carried out the year round. In the north the species does not feed in the late autumn and early winter (Pearse, 1919).

Data on feeding in the different parts of the range may be summarized as follows.

Wisconsin and Illinois

The food of 140 fish, collected in Lake Wingra, Wisconsin, between February and October, and ranging in length from 165 to 200 mm, is given by Pearse (1919). Cladocera made up 33% of the diet; chironomid larvae and pupae, 24%; Amphipoda, 11%; fish, 9%; Ephemeroptera nymphs, 6%; Copepoda, 5%; adult (flying) chironomids, 4%; and Odonata nymphs, 2%. *Chaoborus* was absent, presumably not occurring in this shallow lake (mean depth, 5 ft; maximum depth, 14 ft). The food in Lakes Mendota and Monana resembled that in Lake Wingra.

There proved to be clearcut seasonal differences in food in the Wisconsin lakes. Amphipoda predominated in February and March, Copepoda between February and April, Odonata nymphs in February, Ephemeroptera nymphs in March and August, fish in August, and Cladocera in March, June, and September. Cladocera were the predominant food type in autumn. Little or no feeding was carried out in winter.

The diet in Illinois (Forbes and Richardson, 1920) proved to be similar. In Clear Lake, Iowa, Bailey and Harrison (1945) found the amphipod *Hyalella* very prominent in the diet, along with dipterous larvae and pupae.

Tennessee

In Norris Reservoir, Tennessee, Dendy (1946) found chaoborines and chironomids, and plankton (especially *Leptodora*), to predominate in the diet of 100–300 mm fish in spring and early summer, young fish making up the bulk of the diet in late summer and fall. These findings are of little comparative value, however, as they are based on the number of stomachs containing each kind of food, and neither volumes nor counts of individuals of each are given.

Florida

Reid (1949) analysed the stomach contents of 902 black crappie from Orange Lake, Florida, ranging in size from 31 to 291 mm standard length. He found the fish to fall into two groups on the basis of a natural break in the types of food organisms ingested. A conspicuous decrease in the consumption of Entomostraca occurred at about 100 mm standard length (= 120–125 mm total length). Fish were first taken as food at a body length of about 75 mm (given as 61 mm standard length), and from then on were eaten with increasing frequency.

Reid's data also are only given on the basis of the proportion of fish containing the different food items and hence are not fully comparable with those from Ontario and Wisconsin. He found, however, that Entomostraca occurred in 94% of the stomachs of fish less than 120 mm in length but in only 12% of stomachs of larger individuals. Copepoda made up 84% of the total numbers of entomostracans consumed, and Cladocera 14%. Malacostraca occurred in 44% of crappie stomachs, and 99% of these were *Hyalella*. These latter were absent from fish of less than 70 mm total length.

Insects occurred in 80% of stomachs. Dipterous larvae were found in 33% of all stomachs (41% of those of young individuals, and 32% of adults), and 27% of stomachs contained dipterous pupae and adults (mostly *Chaoborus* and chironomids). Insects other than Diptera occurred in 21%, 78% of these being Odonata nymphs. Ephemeroptera nymphs, Trichoptera larvae, and Coleoptera occurred in small numbers. Only 8% of the crappie of less than 120 mm total length had eaten non-dipterous insects, whereas for the larger fish the figure was 23%.

Fish occurred in 66% of the stomachs and were the most frequently taken food of individuals larger than 125 mm. This frequency implies a considerable predominance in terms of volume. Seventy-four per cent of the adult stomachs, and only 9% of those under 120 mm in length, contained fish. Ninety-nine per cent of the fish eaten were shad, *Dorosoma cepedianum* and *Signalosa petenensis vanhyningi*. Fish consumption reached peaks in May–June and between October and January. Most of the Diptera pupae and adults were eaten in February–March and September, and larvae in February and July–August. Other insects occurred most frequently in March. Entomostraca occurred in most stomachs in June–July, and Malacostraca in January–April. An interesting sidelight was that adult males, guarding nests in January–April, and hence restricted in their feeding range, took a third more malacostracans and a third less shad than the freely ranging adult females.

Texas

The food of crappie in four small lakes (artificial impoundments), Daingerfield, Electro, Ferndale, and Tyng, was studied by Mitchell (MS, 1945). The collections, made in the months of March, July, August, October, and December, show a striking dependence on planktonic Crustacea, even by the larger fish. The volume in an August collection from Daingerfield was 82% in 130–159 mm fish, 46% in 160–189 mm ones, and 35% in those 190–209 mm in length. The most abundant crustacean genera were *Cyclops* (most important in the smallest size-group), *Daphnia*, *Cypris*, and *Diaptomus*. In Electro a March series contained 22% of planktonic Crustacea by volume in 160–189 mm fish and 30% in 250–279 mm fish. The Tyng Lake series averaged volumes of 40% and 12% in 190–219 mm and 220–259 mm fish, respectively. Figures for Crustacea were lower in Ferndale but they amounted to 24% of total stomach volume in March, 42% in July, 17% in October, and 74% in December. Insect larvae predominated in the other months. Fish eating was found to commence in small crappie 60 mm in length but only in fish of 220–250 mm did the volume of fish consumed exceed that of invertebrates. Fish of 300 mm and over proved to be almost entirely piscivorus. The crappie is an introduced species in Texas, having been brought in from the Mississippi Basin (J. G. Silvers, personal communication).

THE TRANSITION FROM PLANKTON TO FISH FEEDING

The food of very young black crappie has been studied in Wisconsin by Pearse (1919) and in Lake Erie by Ewers (1934). The former study shows that *Cyclops* and Cladocera form the major food in the early stages, small insect larvae being of lesser importance. In Lake Erie Copepoda and Cladocera form the exclusive food at first, insect larvae progressively replacing them as the predominant item. This succession is, of course, typical of many large-bodied fish species.

Fish below 60 mm were not included in the Lake Opinicon study. In the 60–115 mm group, however, Crustacea (if, in fact, they were the exclusive food of hatchlings) were already secondary in importance to insect larvae. Fish of 116–160 mm consumed them only during the August–September population peaks of these organisms. The final transition, that to fish-eating, proved to be well-established in the 116–160 mm group but fish only made up a significant proportion of the diet in individuals larger than 160 mm.

The sizes at which these major transitions are established apparently contrast with the situation in Orange Lake, Florida, where the species is already established as predominantly piscivorous at a length of 125 mm, and the one in Texas, where plankton feeding remains prominent in fish 200 mm long. More would have to be known of the associated faunas before the significance of these differences could be determined but it may well represent a basic shift in the food niche at the southern extremity of the range.

The protracted transition from plankton to fish feeding in *P. nigromaculatus* may be contrasted with the very rapid one described by Hunt and Carbine (1950) for the highly specialized piscivore *Esox lucius*. Whereas the transition may take 3 years in the crappie it takes place in 1 or 2 months in the pike. Planktonic Crustacea represent a significant diet item only until pike are 20 mm long. *Pomoxis nigromaculatus* can best be thought of as only "incidentally" piscivorous, taking fish only when the body size is so large that it is uneconomical to take smaller items.

GILL-RAKER FORMULAE OF AND PLANKTON FEEDING IN *P. NIGROMACULATUS* AND OTHER CENTRARCHIDS

The numbers of gill rakers on the various pharyngeal arches of *P. nigromaculatus* are exceptionally large compared with those for the other centrarchids in Lake Opinicon, i.e. 25–29 on the first arch compared with 8–12 in the other cohabiting species: *Ambloplites rupestris*, *Micropterus salmoides*, and *Lepomis macrochirus* (Table III). By contrast the alewife, *Alosa pseudoharengus* (Clupeidae), the other "plankton strainer" in Lake Opinicon, has up to 50–55 gill rakers on the first arch (Hubbs and Lagler, 1957). The conclusion to be drawn from the above is that the large gill-raker count of *P. nigromaculatus* permits it to consume planktonic Crustacea until it reaches a large size, if not throughout life.

Along with their small numbers of rakers, plankton feeding is restricted in the three other centrarchids, as follows.

Young *Lepomis macrochirus*, 21–37 mm long, feed largely on small Cladocera and Copepoda (Turner and Kraatz, 1920). By 50 mm a greater dependence on insects is apparent (Pearse, 1915–16). Cladocera, however, continue to be taken in greater numbers (Ransbottom, MS, 1951). In Lake Opinicon fish of 60–85 mm length, Cladocera and Copepoda accounted for only 13–18% of the volume of stomach contents between May and August, and reached 30% during the cyclical peak of these organisms in September (Keast, 1965). Beyond a body length of 115 mm no planktonic Crustacea were con-

TABLE III. Gill-raker counts (ranges) for four cohabiting centrarchids — Lake Opinicon. Total body lengths are given.

Species	No. of fish	Gill-raker formula			
		Arch 1	Arch 2	Arch 3	Arch 4
Pomoxis nigromaculatus					
50–60 mm	10	25–29	20–23	19–21	14–15
160–210 mm	8	25–28	22–25	20–22	15–18
Ambloplites rupestris					
50–60 mm	15	9–11	7–10	7–9	5–7
160–220 mm	10	9–11	6–11	6–9	5–8
Micropterus salmoides					
50–60 mm	10	10–12	9–10	7–9	6–8
110–160 mm	11	8–10	8–9	7–8	5–7
Lepomis macrochirus					
110–150 mm	15	9–12	8–10	5–7	4–6

sumed. The cessation of plankton feeding at about this growth stage would appear to be fairly general.

In *Ambloplites rupestris*, planktonic Crustacea are prominent in the diet of young fish 23–35 mm long, but are largely replaced thereafter by chironomids and other insect larvae (Nurnberger, 1930). Studies of the 45–70 mm length group in Lake Opinicon gave percentage volumes for Cladocera and Copepoda of 8, 12, 4, 19, and 39 for the months May–September (Keast, 1965). They were absent from the diet of fish longer than 75 mm except that a few were taken in August.

Micropterus salmoides takes Entomostraca (and minute midge larvae) at 30 mm body length, but from 30–50 mm only negligible amounts are consumed (Turner and Kraatz, 1920; Ewers and Boesel, 1935). In Lake Opinicon they are consumed up to a body length of 50 mm, but not beyond (Keast, 1965). In the closely related small-mouth bass (*M. dolomieui*) planktonic Crustacea are important to the 40-mm stage but are absent from fish 50–65 mm in length (Wickliff, 1920).

The attenuated period of plankton feeding in *P. nigromaculatus* in the Texas lakes studied by Mitchell (1945) raises the possibility that these populations have a high gill-raker formula. Counts carried out by Mr Alex E. Peden, University of Texas, through the courtesy of Professor Clark Hubbs, on Texas specimens in the University museum show, however, that the numbers average only slightly higher than in the Lake Opinicon fish. Thus, of 21 specimens ranging from 60 to 160 mm in body length, four had 28 rakers on the first arch, five 29, eight 30, and five 31. A single Florida specimen, measuring 120 mm, had a gill-raker count of 31.

ACKNOWLEDGMENTS

I should like to express my sincere thanks to the following granting agencies for their confidence and support: National Research Council of Canada, the Ontario Research Foundation, and the Canadian National Sportsmen's Show.

A number of people assisted in phases of the food analyses, including R. Dickey and R. Martin. Dr A. L. Hamilton, Entomology Research Institute, Canada Department of Agriculture, Ottawa, kindly identified the chironomids, and Nelson Watson of Queen's University confirmed the identification of the Cladocera. Linda Welsh made the gill-raker counts.

I am indebted to Professor J. K. G. Silvey of North Texas State University for permission to reproduce data from the unpublished M.Sc. thesis of G. C. Mitchell (1945), to Alex E. Peden for carrying out gill-raker counts of Texas and Florida crappie on my behalf, and to Prof. Clark Hubbs for arranging same.

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Effect of Stage of Rigor and of Freezing–Thawing Processes On Storage Quality of Refrozen Cod[1]

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ABSTRACT

Trawler-caught cod were frozen before and after rigor mortis in brine (23% NaCl) and between refrigerated plates, thawed in circulating water at 7 C or in a conveyorized microwave oven, then processed into fillets which were packaged, plate-frozen, and stored at −18 C.

All samples thawed satisfactorily in circulating water. Some overheating was encountered during thawing in microwaves. But the equipment was not developed sufficiently to permit assessment of the commercial potential of microwave thawing.

Examinations of the fillets from the thawed fish for appearance, odor, and texture showed that freezing pre-rigor is preferable to freezing post-rigor and that thawing by means of microwaves is preferable to thawing by means of water. Freezing or thawing methods did not affect the *p*H of the thawed fillets.

Results of organoleptic and chemical tests to determine the changes in quality of the refrozen fillets packaged and stored at −18 C for 12 months indicated that neither the average taste panel scores nor the chemical tests for moisture, total lipid, free fatty acids, and extractable protein nitrogen showed any difference attributable to state of rigor, freezing method, or thawing method. The taste panel slightly preferred the texture of fillets from fish frozen pre-rigor and from fish frozen in brine. Free fatty acids increased sharply as a result of thawing and refreezing, and the rapid increase continued during the first 2 months of frozen storage. Taste panel scores correlated significantly with free fatty acids (1% level) and with extractable protein (5% level).

INTRODUCTION

Some members of the fishing industry in Newfoundland and Quebec have sought to freeze along the Labrador coast or on the Grand Bank such quantities of eviscerated, eviscerated and headed, or whole groundfish as may be later thawed, filleted, and marketed unfrozen or refrozen in an economic operation. The Fisheries Research Board of Canada was charged with the responsibility to investigate the actual freezing, storing, thawing, and refreezing phases of the operation and to spell out, where possible, the effects of various treatments on the quality of the double frozen pack. In attacking this problem, it became apparent that the following were important variables:

(1) *State of rigor mortis.*— Knowledge of the best condition for freezing would be helpful because fish are presently available to freezing vessels and to fish plants in various states of rigor. Banks (1962) states that required firmness can be obtained in thawed, eviscerated trawler cod if storage in ice,

[1]Received for publication September 23, 1966.

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J. Fish. Res. Bd. Canada, 25(2): 299–320, 1968.
Printed in Canada.

prior to freezing, is limited to 3 days. But eviscerated Newfoundland trap cod frozen and thawed from catches iced 3–4 days were softer than were fish frozen before rigor mortis (W. A. MacCallum and D. G. Ellis, unpublished data). Other results (unpublished) obtained at the Fisheries Research Board of Canada, St. John's, Nfld., Technological Unit also showed that trap cod should not be kept in ice more than a day or so prior to processing; Fougere observed noticeable flaking in fillets held longer than 4 days in ice, whereas taste panel results (E. J. Laishley and W. A. MacCallum, unpublished data) showed the texture of fillets frozen before rigor to be significantly better than that of frozen fillets prepared from cod held 4 days in ice. Also, based on taste panel assessment, MacCallum et al. (1966) found frozen stored fillets prepared in June from iced trap fish before and during rigor to be of equal quality. They found too that freezing (both before and during rigor), thawing, refreezing, and storage of June trap fish longer than a few weeks resulted in poor but still acceptable quality; July trap fish were of similar quality immediately after single and double freezings, both packs graded only fair. The quality of Grand Bank cod varied much more due to time and place of catching than to state of rigor at freezing. Both pre- and in-rigor October catches, once- and twice-frozen, were of only fair quality soon after freezing, but cod captured in March and frozen after rigor were of initial high quality when frozen once and twice, and remained for many months in a very acceptable condition. Other recent work has been reviewed by Jones (1965) who concluded that although handling conditions are very important, fish should be frozen before or during rigor.

(2) *Freezing method.*— Of the four major freezing methods — immersion (in sodium chloride brine), refrigerated plate, blast, and shelf coil — the first two have been most commonly used in freezing-at-sea applications (Eddie, 1962; Peters and Slavin, 1960; Slavin, 1960). Each has its advantages and disadvantages, but research has not been undertaken to weigh each method objectively and permit a rational choice of the one most satisfactory for this or similar applications.

(3) *Thawing method.*— Fish should be thawed under controlled conditions to permit further processing and packing in blocks or 1-lb packages for re-freezing. Electronic thawing methods using microwave or dielectric energy will supply heat rapidly and fairly uniformly throughout a mass of frozen fish, and the dielectric method has been reported suitable for thawing blocks of frozen fish (Jason and Sanders, 1959, 1962a, b; Bengtsson, 1964; MacCallum, 1964). However, the value of the process for thawing Newfoundland blocks has been questioned (Anon., 1967); to avoid overheating, these blocks could be only partially thawed dielectrically followed by complete thawing in water. Early in 1962, dielectric equipment was not available to the authors but it was possible to borrow a conveyorized microwave unit from the Raytheon Company, Waltham, Mass. The opportunity to examine microwave thawing was welcomed since, technically, it appeared that the method might have three advantages in reduction or elimination of corona discharge, in promoting

relatively rapid thawing, and in the production of relatively compact equipment. The authors expected that the tests would have to be conducted at some disadvantage to the microwave method as the oven available could accommodate only blocks of up to 1½ inches thick and there was no provision for microsecond exposures to microwave energy interrupted by longer energy-off periods. There was also an apparent disadvantage posed by the then relatively high cost of magnetrons (for replacement), bringing the estimated tube replacement and power costs of thawing by the microwave method to about $0.0058 per pound of fish. Jason and Sanders (1962b) estimate for the same items a cost of $0.00106 for each pound of fish thawed in a dielectric apparatus operating 16 hr per day.

Consideration was given to circulating humidified warm air for thawing the blocks as this method was used successfully in Great Britain (Waterman, 1963; Merritt and Banks, 1964), but the water-thawing method was chosen in view of the experience already available on thawing block-frozen (MacCallum and Ellis, 1964) and individually frozen (Magnusson, 1952; Magnusson and Hartshorne, 1952; Peters and Slavin, 1960; W. A. MacCallum, unpublished results of the FRBC, St. John's, Nfld. Technological Unit's 1961 Research Program) fish in water, and on refreezing and storing the finished fillets (Dyer et al., 1962; Peters and Slavin, 1960).

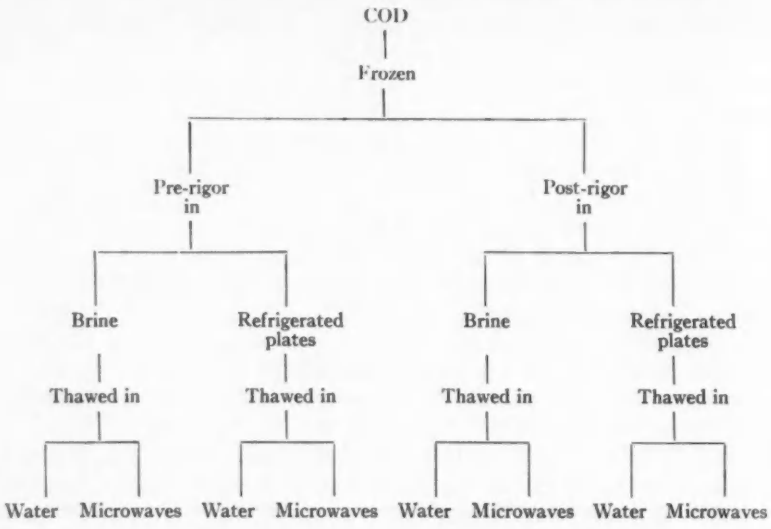
(4) *Storage temperatures.*— For the refrozen fish a temperature of −18 C was selected as a commercially used temperature where storage changes would become apparent in a relatively short time. Quality of fish after refreezing is usually good but may or may not deteriorate rapidly on storage (Dyer et al., 1962; MacCallum et al., 1966).

The following study was therefore undertaken to answer the above questions and to provide information on methods for freezing and thawing Bank cod. It represents a cooperative effort between the Fisheries Research Board of Canada's Technological Unit at St. John's, Nfld., and the Halifax Laboratory, Halifax, N.S., and the U.S. Bureau of Commercial Fisheries Technological Laboratory, Gloucester, Mass.

The specific objectives of this study were: (1) To define the effect on quality of (*a*) freezing fish in the pre- or postrigor condition; (*b*) freezing in brine or by refrigerated plates; (*c*) thawing in water or by microwave energy. (2) To determine the effect of the above factors on the subsequent storage life of the refrozen fillets. (3) To determine the feasibility of using microwave energy to thaw fish on a commercial scale.

PREPARATION OF THE TEST SAMPLES

The cod used in the tests shown in the experimental design (Scheme I) were obtained off Sable Island by the Fisheries Research Board vessel *A. T. Cameron* in a 30-min haul on March 14, 1962. About 1000 lb were gutted and iced in the hold, and about the same weight of fish was kept alive in four tanks on deck. Upon being landed at Halifax 7 hr after capture, 350 lb of live scrod cod



SCHEME I. Experimental design for collaborative project.

was gutted, headed, and iced. Within an hour of icing, 38–40 lb of fish were packed in each of six aluminum alloy pans measuring 27 × 17 × 3 inches (stowage density 47.5–50 lb per ft³). The pans were covered with aluminum alloy sheet and placed between the plates of a horizontal plate freezer, operating with plate temperatures in the range of −33 to −39 C.

The remaining live scrod and market size cod were held for 2 days at 4 C in recirculating seawater, then gutted, headed, and iced for prerigor brine freezing. Fish were packed in three levels in two rectangular wire baskets measuring about 37 × 7 × 26 inches (stowage density, 30 lb per ft³). Wire spacers provided head room for each layer, ensuring that the fish did not clump together and slow the rate of freezing. Three fish in each basket were fitted with thermocouple leads. The baskets were suspended in a fibreglass reinforced plastic tank 6 ft in diameter × 4 ft deep (capacity, 840 U.S. gal) with the fish submerged in 23% NaCl brine. The brine was refrigerated directly to about −17 C by means of a high efficiency ammonia coil. Circulation of brine across the coil and the fish was maintained by a ¼ hp electric stirrer (420 rpm).

Three days after capture the iced fish, then in postrigor state, was divided into three lots, two of which were used for freezing between plates or in brine as described above and the third for filleting and freezing as controls.

In the four freezing exercises (pre- and post-rigor, plate- and brine-frozen) using unfilleted fish, temperatures within the fish, in the brine, and on the freezing plates were recorded using copper-constantan thermocouples and a multipoint recorder. After freezing the four lots of cod were coded, wrapped

in 8-mil polyethylene sheet, and stored at −26 C in the laboratory. Control samples (fillets) were processed at a commercial plant, packed in separate 1-lb waxed cartons, overwrapped with lacquer-coated cellophane, frozen, and stored at −26 C in the laboratory.

All samples other than controls were packed in foam-plastic insulated wooden boxes, refrigerated with dry ice, and shipped in the refrigerated holds of the M/S *Aalesund* on March 30. They were unloaded in Gloucester 4 days later and transferred to a −29 C storage room in the laboratory. Temperature on arrival was −23 C. Control samples were shipped in light-weight, insulated, dry-ice refrigerated containers by air freight from Halifax to Boston and transferred immediately to the Gloucester Laboratory. Temperature on arrival was −26 C. Storage thereafter was at −29 C.

THAWING

Plate-Frozen Samples

The 27 × 17 × 3 inch blocks of cod, weighing about 40 lb each and frozen pre-rigor and post-rigor, were thawed in circulating tap water at 7 C in separate batches in a stainless steel tank arranged to support the blocks by the method described by MacCallum and Ellis (1964). The fish were separated from the block as soon as possible, then left in the tank until thawing was complete.

It was easier physically to separate the individual fish from the prerigor blocks, and they separated earlier; 1 hr and 10 min as compared with 1 hr and 35 min for the post-rigor. In either case thawing was considered complete in 3 hr, although some of the larger fish were still slightly frozen in the center when filleted.

Plate-frozen fish were easy to filiet, but the fillets from the fish frozen post-rigor flaked more easily than did those frozen pre-rigor.

In preparation for the microwave thawing tests, 27 × 17 × 3 inch blocks of cod were cut into slabs measuring 27 × 11 ½ × 3 inches and 27 × 5½ × 3 inches, and then split to a thickness of 1½ inches. In operation, one of the larger slabs, or two of the smaller slabs side by side, were fed through the machine to load the belt fully. After preliminary tests in which various belt speeds and numbers of passes through the cavity were tried (see App. I) it was decided to expose the test slabs to microwave energy for 1 min at each pass for a total of five passes. At the end of each pass, the slabs were turned end for end and upside down to permit as uniform thawing as possible.

Brine-Frozen Samples

For thawing the brine-frozen fish in tap water, the tank was divided lengthwise into two equal sections by an expanded metal screen so that both the pre- and postrigor samples (total weight about 240 lb) could be thawed at the same time.

The brine-frozen fish were generally larger than the plate-frozen fish, and the largest of these were difficult to fillet since they were still frozen in the center. The fillets from the brine-frozen fish were firmer and flaked less easily than did the fillets from the plate-frozen fish.

Although the brine-frozen fish lost their initial buoyancy and there was about twice the weight of brine-frozen fish as plate-frozen fish thawed at one time, the thawing rates for the brine- and plate-frozen samples were very similar.

Typical temperatures at the time the fish were removed from the tank were: center = −1.5 to −2.2 C; 1 inch in from the surface = 5.5 C; and at the surface = 7–9 C.

Prior to thawing in the microwave unit, the individual brine-frozen fish were split lengthways to a maximum thickness of 1½ inches. The pieces were packed close together on the belt to keep a continuous stream flowing under the magnetrons. Other conditions of thawing were as described for the plate-frozen fish.

EXAMINATION OF THE THAWED FISH

After being thawed and filleted, the various test samples were examined for appearance, odor, and texture by a five-member panel, and each attribute was rated on a five-point scale ranging from 5 for excellent quality to 1 for poor quality.

Also, the *p*H of the skin surface and cut surface of the fillets was determined with a glass electrode.

REFREEZING AND EVALUATING THE THAWED SAMPLES

Preparation

The samples were packed in ice immediately after being thawed. The fish were then filleted, skinned, and packed in 1-lb waxed paperboard cartons overwrapped with waxed bleached sulfite paper and were refrozen in a horizontal plate freezer. After freezing, the packages were transferred to a −18 C cold room at the Gloucester Technological Laboratory, where they were held for a 12-month storage test. Taste tests were conducted on the various refrozen samples to determine changes in quality occurring during the 12-month storage period.

Organoleptic Evaluation

The samples were removed from the −18 C storage room, cut into uniform sized pieces, put in stainless steel pans, covered tightly with aluminum foil, and steamed for 25 min. No sauces or condiments were used. The control samples, which had been stored at −29 C during the 12-month period, were prepared in the same manner as were the test samples.

The cooked samples were served to a 10–12 member panel made up of personnel experienced in the testing of fishery products. At each taste session, four portions were served simultaneously. One identified control and one unidentified or blind control served as a check on the sensitivity of the panel members. The other two portions were from the test samples and were paired for each test by state of rigor and freezing method. The panelists rated each

sample for the attributes of appearance, odor, flavor, texture, and overall quality on a nine-point scale ranging from "excellent" to "inedible."

Single taste tests were run on each sample at the 0- and 1-month periods. All other tests were run in duplicate.

Preparatory to statistical analysis of the data, individual score sheets were examined and, if a panelist's average score for the blind control differed by more than 1.2 score units from his average score for the known control, his results were eliminated from further calculations.[6] The Q test (Dean and Dixon, 1951) was used to eliminate the results of a panelist whose average score for any one sample varied excessively from the average scores of the other panelists.

At each test period and at the conclusion of the test, the average scores for the various samples were examined by analysis of variance, and Student's *t*-test (Snedecor, 1946) was used to determine the significance of difference between average scores for the various attributes of the test samples.

Chemical Tests

To supplement the taste-panel data, changes in the moisture, protein, and lipids were measured at the Halifax Laboratory in iced, unfrozen, post-rigor fillets, in round fish frozen 1 week, and in the refrozen fillets at 2-month intervals up to 1 year, and on control once-frozen fillet samples stored at −29 C. The stored samples were shipped by air express from Gloucester to Halifax for analysis. They were packaged in cardboard cartons lined with 1-inch expanded polystyrene insulation, containing slabs of dry ice separated from the packaged frozen fillets by a layer of cardboard. Temperatures on arrival varied from −34 to −40 C.

For analysis, cross sections of the fillet block were diced and representative mixed samples were weighed and analysed immediately, without allowing them to thaw.

Moisture was determined by drying samples of about 20 g to constant weight at 105 C, usually 24–48 hr.

Protein nitrogen extractable in 0.6 M NaCl (EPN) was measured by the procedure of Dyer et al., (1950, 1964); results of duplicate extractions are recorded. Tissue lipid was extracted quantitatively by the phase separation procedure of Bligh and Dyer (1959), and free fatty acid (FFA) was determined as described by Bligh et al. (1957), assuming a mol wt of 300.

RESULTS

Quality of the Thawed Fish

Comparisons of the ratings for appearance, odor, and texture of the various samples show that (1) the fish frozen pre-rigor rated considerably higher than those frozen post-rigor, especially in the scores for appearance and

[6]This procedure is based on analysis of numerous taste-panel scores at the Gloucester Technological Laboratory which showed agreement between the known and blind controls to be within 1.2 score units better than 95% of the time (J. A. Peters, unpublished data).

texture; (2) the freezing method had no great effect on quality; and (3) the fish thawed in microwaves rated considerably higher in appearance and texture, and slightly higher in odor, than did those thawed in water (Tables I and II).

TABLE I. Average quality scores of the raw fillets.

Treatment (other than thawing)	Thawing method							
	Water				Microwave			
	Appearance	Odor	Texture	Avg	Appearance	Odor	Texture	Avg
Pre-rigor								
Brine frozen	3.2[a]	4.7	3.4	3.8	3.9	4.7	4.2	4.3
Plate frozen	3.7	4.9	3.6	4.1	5.0	5.0	5.0	5.0
Post-rigor								
Brine frozen	3.5	4.5	3.4	3.6	3.6	4.7	3.6	4.0
Plate frozen	2.2	4.3	2.2	2.9	3.5	5.0	4.0	4.2
Avg	3.2	4.6	3.2	3.7	4.0	4.9	4.2	4.4

[a]Scores based on 5 = excellent, 4 = very good, 3 = good, 2 = fair, and 1 = poor.

TABLE II. Quality scores of raw fillets averaged by treatment.

Treatment	Appearance	Odor	Texture	Grand avg of attributes
Pre-rigor frozen	4.0[a]	4.8	4.1	4.3
Post-rigor frozen	3.3	4.6	3.3	3.7
Brine frozen	3.7	4.7	3.7	4.0
Plate frozen	3.7	4.8	3.7	4.1
Water thawed	3.2	4.6	3.2	3.7
Microwave thawed	4.0	4.9	4.2	4.4

[a]Scores based on 5 = excellent, 4 = very good, 3 = good, 2 = fair, 1 = poor.

Particular observations made were that (1) fillets from the post-rigor frozen fish flaked more easily than did those from the pre-rigor frozen fish; (2) fillets from water-thawed fish showed a slight brown discoloration, particularly on the skin side of the fillets, which was absent from the fillets from microwave-thawed fish; and (3) the fillets from fish frozen pre-rigor were somewhat browner than were those from fish frozen post-rigor regardless of freezing method used. The discoloration was not, however, sufficient to cause a downgrading in the appearance of the fillets.

Comparison of the average *p*H's for the various samples shows that the prerigor fish were slightly higher than the postrigor, and the skin surfaces were lower than the cut surfaces (Table III). Freezing or thawing methods did not affect the *p*H of the thawed fillets.

TABLE III. *p*H of thawed fillets.

Treatment (other than thawing)	Thawing method			
	Water		Microwave	
	Skin surface	Cut surface	Skin surface	Cut surface
Pre-rigor	6.5	6.8		
Brine frozen	6.8	6.8	6.8–7.0	6.8–7.0
Plate frozen	6.5	6.7–6.8	–	–
Post-rigor	6.6	6.8–6.9	6.2	6.3–6.4
Brine frozen	6.3–6.5	6.4–6.5	6.5–6.6	6.6–6.8
Plate frozen	Firm 6.4 Soft 6.6–6.7	6.5–6.6 6.6–6.8	–	–

ORGANOLEPTIC EVALUATION OF THE FROZEN SAMPLES

The averages of the taste-panel scores for the attributes of appearance, odor, flavor, texture, and overall quality for the various samples during the 12-month test period are given in Table IV, and the taste-panel scores averaged by treatment are given in Table V.

Slight dehydration and discoloration of the test samples had occurred by the 6th month of the test and became moderate to severe by the 8th month.

TABLE IV. Summary of taste-panel scores.[a]

Sample	Storage time (*months*)								
	0	1	2	4	6	8	10	12	Avg
Plate									
Pre-water	8.0	7.0	6.8	6.0	5.7	5.8	6.7	6.2	6.5
Pre-micro	7.8	6.1	6.6	5.9	5.7	5.2	6.7	5.6	6.2
Post-water	7.9	6.4	6.4	5.4	6.1	6.1	6.7	5.8	6.4
Post-micro	7.3	6.2	6.6	5.8	5.5	6.1	6.4	5.7	6.2
Brine									
Pre-water	7.2	6.2	6.5	5.8	6.1	5.6	6.8	6.1	6.3
Pre-micro	7.3	6.8	6.8	6.0	6.1	5.8	7.1	6.7	6.6
Post-water	8.2	6.3	6.5	6.1	5.5	5.5	6.4	6.3	6.4
Post-micro	7.7	5.9	6.6	6.1	5.5	5.3	6.8	6.5	6.3
Avg — test samples	7.7	6.4	6.6	5.9	5.8	5.7	6.7	6.1	6.4
Controls									
Known	8.4	7.7	7.8	7.8	8.0	7.9	7.8	7.4	7.9
Blind	8.0	7.0	7.5	7.5	7.7	7.7	7.6	7.2	7.5

[a]Scale used was: 9 = excellent, 8 = very good, 7 = good, 6 = fair, 5 = borderline, 4 = slightly poor, 3 = poor, 2 = very poor, 1 = inedible.

TABLE V. Average taste-panel scores[a] by treatments.

Sample treatment	Storage time (*months*)								
	0	1	2	4	6	8	10	12	Avg
Plate frozen	7.8	6.4	6.6	5.8	5.8	5.8	6.6	5.8	6.3
Brine frozen	7.6	6.3	6.6	6.0	5.8	5.6	6.8	6.4	6.4
Pre-rigor frozen	7.6	6.5	6.7	5.9	5 9	5.6	6.8	6.2	6.4
Post-rigor frozen	7.8	6.2	6.5	5.9	5.7	5.8	6.6	6.1	6.3
Water-thawed	7.8	6.5	6.6	5.8	5.9	5.8	6.7	6.1	6.4
Microwave thawed	7.5	6.3	6.7	6.0	5.7	5.6	6.8	6.1	6.3
Avg	7.7	6.4	6.6	5.9	5.8	5.7	6.7	6.1	6.4
Controls									
Known	8.4	7.7	7.8	7.8	8.0	7.9	7.8	7.4	7.9
Blind	8.0	7.0	7.5	7.5	7.7	7.7	7.6	7.2	7.5

[a]Scale used was: 9 = excellent, 8 = very good, 7 = good, 6 = fair, 5 = borderline, 4 = slightly poor, 3 = poor, 2 = very poor, 1 = inedible.

The cause was traced to faulty operation of the expansion valves in two of the five banks of refrigerated plates in the storage room, which resulted in considerable decrease in relative humidity in the room, and accelerated the escape of water vapor through the carton and overwrap. For the 10- and 12-month tests the dehydrated surfaces were trimmed from the samples prior to cooking, probably accounting for the sharp increase in taste-panel scores at the 10-month test. This increase, however, would not mask differences among the samples resulting from pre-freezing treatment, since it occurred with all samples.

Analysis of the average taste-panel scores shows that the test samples had become significantly different from both controls by the 6th month of storage (at subsequent test periods occasional test samples were not significantly different from the blind control, but all were different from the known control). Under proper storage conditions it is probable that the time to the first detectable difference between the controls and the test samples would be considerably longer than the 6 months found in this study. Although there was some variation among the average scores for the various test samples at any one test period, the differences were not consistent, and the average scores for the whole test period showed no significant difference attributable to state of rigor, freezing method, or thawing method.

The present results may be compared with those reported by MacCallum et al. (1967) who conducted storage tests on double frozen cod and redfish. These authors found both trap-caught and Grand Bank cod thawed in tap water at 7.2 C and 15.5 C to be of similar quality. Also, the storage quality of trap fish and Grand Bank redfish was influenced equally by thawing in tap water at 15.5 C or dielectrically.

Comparison of the average scores given by the panel during the 12-month test to the various samples for the attributes of appearance, odor, flavor,

texture, and overall quality showed that there was a very slight but extremely consistent preference for the texture of samples frozen pre-rigor (average score = 6.29) as compared with those frozen post-rigor (average score = 6.04), and that this preference proved to be significant at the 2% level. An equally consistent but even slighter preference was shown for the texture of the brine-frozen samples over the plate-frozen samples (average scores 6.21 and 6.11 respectively), and this preference proved to be significant at the 5% level. There were no differences in the other attributes that could be related to treatment.

Results and Discussion of Chemical Tests

The values found for moisture, lipid, FFA, and EPN are given in Table VI.

Table VI. Moisture, extractable protein nitrogen (EPN), and lipid as per cent wet weight and free fatty acid (FFA) as per cent of lipid, in iced round frozen, and in refrozen cod fillets prepared from cod frozen in prerigor or postrigor condition by refrigerated plates or by brine immersion, and thawed in circulating water or by microwave energy.

Sample[a] and analysis	Not frozen	Frozen round 1 wk	Refrozen fillets (*months*) −18 C 1 wk	2	4	8	10	12	Avg
Moisture %									
Plate — Pre-water			81.1	81.2	81.0	81.6	81.1	81.9	81.3
Pre-micro			82.1	81.7	81.2	81.6	81.1	80.6	81.4
Post-water			83.0	82.2	83.6	81.5	81.8	82.4	82.4
Post-micro			82.5	81.5	82.4	81.9	81.4	81.6	81.9
Brine — Pre-water			81.6	80.4	80.7	80.1	80.8	79.8	80.6
Pre-micro			80.0	80.0	81.8	79.7	79.4	79.7	80.1
Post-water			81.6	81.5	80.7	81.0	81.7	81.0	81.3
Post-micro			82.3	80.9	81.9	81.4	80.9	81.2	81.4
Avg			81.8	81.2	81.7	81.1	81.0	81.0	81.3
Control			81.8	82.0	82.3	82.8	82.4	82.1	82.2
EPN %	2.29								
	2.22								
Plate — Pre-water		2.28	2.19	1.90	1.69	1.31	1.07	1.13	
		2.24	2.22	1.86	1.68	1.26	1.23	1.17	
Pre-micro			2.21	1.93	1.64	1.05	0.90	0.64	
			2.19	1.88	1.83	1.28	0.91	0.56	
Post-water		2.18	2.06	1.98	1.75	1.20	1.03	0.65	
		2.14	2.09	1.84	1.66	1.02	1.08	0.63	
Post-micro			1.83	1.69	1.63	0.78	1.51	0.67	
			1.95	1.70	1.74	1.29	1.51	0.65	
Brine — Pre-water		2.29	2.19	1.83	1.83	0.86	1.23	1.02	
		2.25	2.10	1.70	1.89	0.83	1.01	0.95	
Pre-micro			2.19	1.61	1.18	1.43	1.21	0.81	
			2.12	1.78	1.19	1.32	1.15	0.81	
Post-water		2.02	2.07	1.74	1.57	1.58	0.99	0.86	
		2.06	2.08	1.70	1.51	1.54	1.03	0.81	
Post-micro			1.95	1.93	1.77	1.17	1.19	1.22	
			1.98	1.89	1.62	0.98	1.22	1.28	

(*Continued*)

TABLE VI. Moisture, extractable protein nitrogen (EPN), and lipid as per cent wet weight and free fatty acid (FFA) as per cent of lipid, in iced round frozen, and in refrozen cod fillets prepared from cod frozen in prerigor or postrigor condition by refrigerated plates or by brine immersion, and thawed in circulating water or by microwave energy. — (*Concluded*)

Sample[a] and analysis	Not frozen	Frozen round 1 wk	Refrozen fillets (*months*) –18 C 1 wk	2	4	8	10	12	Avg
EPN % (cont'd)									
Avg	2.26	2.18	2.09	1.81	1.64	1.18	1.14	0.87	
Control		1.93		1.95	2.07	2.01	1.99	1.88	
		1.93		1.85	2.08	1.99	1.93	1.88	
		2.01							
		2.05							
Lipid %	0.77								
Plate — Pre-water		0.82	0.72	0.58	0.69	0.62	0.69	0.63	0.65
Pre-rigor			.63	.64	.63	.59	.60	.69	.63
Post-water		.82	.66	.66	.59	.68	.67	.70	.66
Post-micro			.67	.73	.67	.83	.68	.64	.70
Brine — Pre-water		.93	.66	.75	.51	.68	.72	.85	.69
Pre-micro			.82	.70	.63	.67	.65	.66	.69
Post-water		.74	.81	.70	.68	.84	.69	.73	.74
Post-micro			.70	.68	.63	.71	.71	.69	.69
Avg		0.83	0.71	0.68	0.63	0.70	0.68	0.70	0.68
Control		.74							
		.74		.66	.69	.76	.78	.69	0.72
FFA %	8.6								
Plate — Pre-water		7.3	17.9	40.7	50.2	43.6	49.4	57.2	
Pre-micro			13.8	39.0	32.9	47.6	50.0	46.9	
Post-water		8.8	15.7	35.8	44.6	44.6	41.9	48.8	
Post-micro			16.1	34.2	33.8	39.9	44.5	53.4	
Brine — Pre-water		8.4	14.9	36.0	46.3	45.2	44.6	46.1	
Pre-micro			13.7	41.1	41.7	45.2	48.8	51.9	
Post-water		10.1	16.4	45.9	33.3	39.5	38.4	48.0	
Post-micro			15.0	36.8	36.2	45.2	46.3	44.0	
Avg		8.7	15.4	38.7	39.9	42.9	45.5	49.5	
Control		9.2		18.8	16.4	17.8	19.8	25.4	
		9.2							

[a]Sample treatment: unfrozen fillets analyzed after 5 days in ice; frozen round fish were stored at about –26 C prior to thawing, filleting, packaging, and refreezing with subsequent storage at –18 C. Control fillets were processed from the same lot of fish and stored at –29 C.

Moisture.— The values for moisture in the refrozen samples decrease slightly during storage, averaging 81.3%, whereas the control stored at a lower temperature, –29 C, and wrapped in coated cellophane does not appear to change and averages 82.2%. No difference in moisture content of the refrozen fillets is evident between those thawed in water or by microwaves. The averages further

show that the postrigor plate-frozen samples tend to be higher and the prerigor brine-frozen samples lower than the remainder of the samples (Fig. 1). The control sample also shows a high moisture level, 82.2%. Calculations of significance show that the refrozen fillets from the plate-frozen postrigor fish

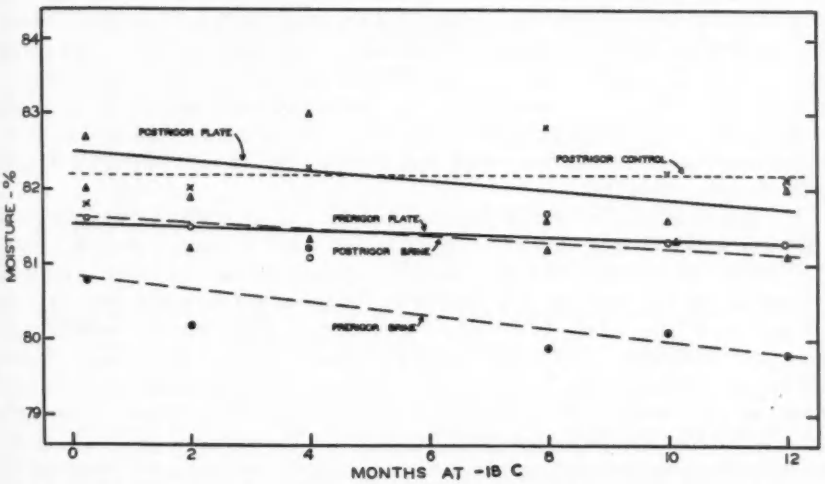


FIG. 1. Moisture content of refrozen samples during storage at –18 C and control fillets at –29 C. Values for water and microwave thawing are averaged. × — control; ○ — prerigor — plate frozen; ● — prerigor — brine frozen; △ — postrigor — plate frozen; ▲ — postrigor — brine frozen.

have a significantly higher moisture content than the fillets from the brine-frozen prerigor fish, but no other differences appear (5% level). The control sample, once frozen as fillets, is similar to the plate-frozen postrigor sample indicating no effect of thawing and refreezing, and is significantly higher at the 1% level than the brine-frozen pre-rigor fish; it is also higher than the plate-frozen prerigor sample and the brine-frozen postrigor sample at the 5% level.

Although there appears to be a trend for the samples to dry out on storage, calculation of averages for each months sampling shows no significant change on storage, differences between samples being greater than those caused by storage changes.

Several factors could exert an influence on the moisture content of the refrozen fillets. During icing of the gutted fish there is usually a loss of moisture, though this may not be large during the early stages or in thin layers (Ellison, 1934). The size of the fish and its biological condition could appreciably influence moisture content (Love, 1960). During freezing in strong brine (23% salt) there may be a slight drying of the surface, which should not greatly affect the fillet. Thawing in water or in the microwave thawer apparently had little effect since there were no differences in the finished product attributable to this factor.

Moisture pickup during washing of the fillets prior to packing can be appreciable (Castell, 1967). In the present work the fillets prepared for refreezing were packed and frozen a few minutes after washing and little opportunity for draining off the absorbed moisture occurred; thus, this factor may well be the major source of the moisture differences. During packing it was noted that the plate-frozen samples were very soft and this could result in considerable moisture uptake on washing. Although not as soft as the plate-frozen ones, the postrigor brine-frozen samples were softer than the corresponding prerigor fillets, an observation in agreement with the final moisture distribution. Also in the prerigor frozen fish, if there is any tendency to thaw rigor development, these fish would lose moisture rather than absorb it during washing after thawing.

Total lipid.— The total lipid content (the washed residue from the first extraction was resuspended and extracted with fresh solvent) averages 0.68% (±0.0097) (SE mean for 48 samples) in the various refrozen samples, averages of treatments ranging from 0.63 to 0.74%, variations between sampling periods being about the same as between treatments. The control sample was also not different, 0.72% in agreement with values previously found (Bligh and Dyer, 1959; Fraser et al., 1961). One initial value on refrozen, postrigor fish was 0.77% and the round fish frozen 1 week averaged 0.83 (±.04) for four samples, somewhat higher than the refrozen samples. Some change resulting in a reduced lipid extractability seems to have occurred in the refrozen samples. The increasing lipid hydrolysis, to 50%, has not, however, affected the degree of extractability.

Free fatty acids.— The free fatty acid levels of the unfrozen, round frozen fish, and control fillets lie in the range usual for good quality fish found by W. J. Dyer and D. I. Fraser (unpublished) but higher than 1.5–5.0% for unfrozen cod reported by Olley and Lovern (1960). The values show little change on freezing but almost doubled on thawing, processing, and refreezing followed by storage at –18 C for 1 week (Fig. 2), increasing from an average of 8.7 to 15.4%. A major increase, however, occurred between 1 week and 2 months' storage of the refrozen fillets at -18 C, FFA values increasing to an average of 38% with a further slow increase to 49% at 12 months. This finding is in agreement with previous results (Dyer et al., 1962; MacCallum et al., 1964) where it was observed that there was an accelerated lipid hydrolysis after thawing and refreezing, or simply raising the temperature of the fish to –12 C or above. The results show that the amount of this increase is greater at –12 and –18 than at –23 C. Thus, there is reason to suspect that this is due to an enzyme activation, or, more likely, to a disruption of the membranes or other tissue structures by the melting of the ice occurring during the temperature rise and the resulting accelerated diffusion of reactants to active sites where chemical or enzymic reactions can occur (Dyer, 1967).

The control, once-frozen fillets stored at –29 C, show only a slow increase in FFA over the 12-month storage period as was previously observed in cod fillets at –29 C (Dyer and Fraser, 1959). Inspection of the results do not indicate any differences in FFA due to the various treatments (5% level).

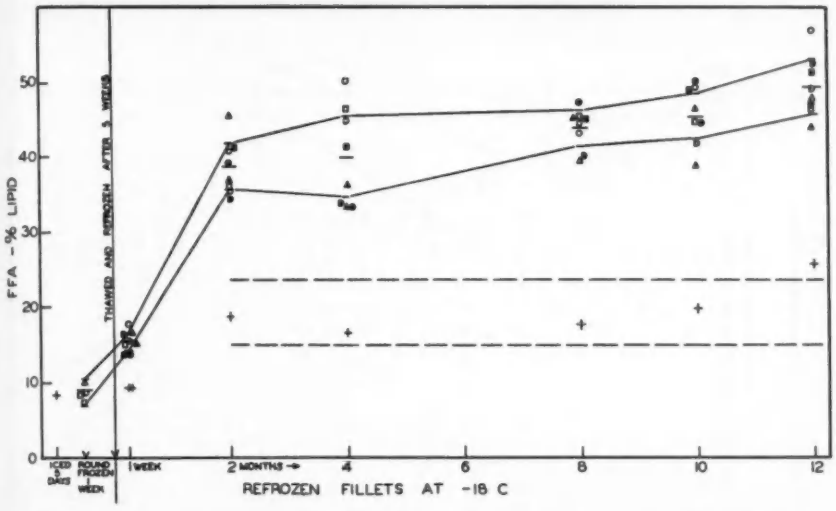


FIG. 2. Free fatty acid as per cent of lipid in postrigor, iced round fish 5 days on ice, in the round fish stored frozen 1 week at –26 C, in the thawed and refrozen fillets stored at –18 C (after having been stored in the round for 5 weeks at –26 C), and in control fillets from the same lot of fish after 3 days, packaged, frozen, and stored at –29 C. Points for individual duplicate determinations are shown along with the 95% confidence interval for the mean of all treatments at each sampling time. For the controls, 95% confidence intervals for average of points at 2–12 months are shown by broken lines. Plate frozen: prerigor water thaw — ○; prerigor microwave thaw — ●; postrigor water thaw — ◗; postrigor microwave thaw — ◗. Brine frozen: prerigor water thaw — □; prerigor microwave thaw — ■; postrigor water thaw — △; postrigor microwave thaw — ▲. Control: +.

Comparison of average FFA values for all test samples with average taste-panel scores for all test samples during the first 8 months show a coefficient of correlation of –0.974, which is significant at the 1% level (Kramer and Twigg, 1962). There is no significant correlation between taste-panel scores on the control samples and FFA content of the control samples.

Extractable protein nitrogen.— The extractable protein, including the proteins of the actomyosin group as well as the water extractable albumins, shows very little decrease on freezing and on thawing and refreezing as fillets (analysed after 1 week at –18 C). This finding agrees with results on trap cod reported previously (Dyer et al., 1962) and also with results on Grand Bank cod found by MacCallum et al. (1966).

On storage at –18 C there is a slow decrease from the fresh-frozen values to very low extractability of the actomyosin at 12 months (Fig. 3). There appears to be little difference due to treatment; control fillets at –29 C do not change throughout; the EPN values average 1.97% with a standard error of 0.019 on 14 determinations over a period of 12 months. This corresponds to a standard deviation of only ±3.5% for variation in a single determination. The variability in results by this procedure is thus very much less than that

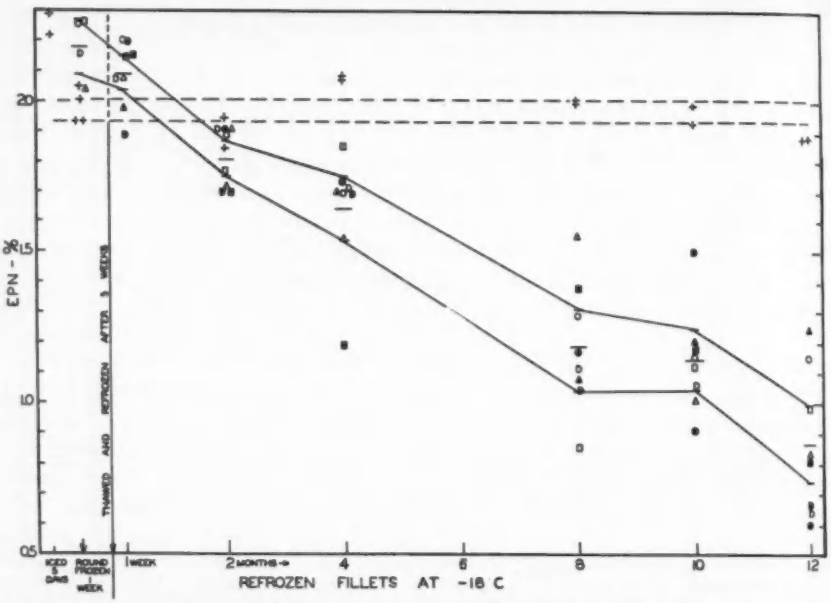


FIG. 3. Extractable protein nitrogen as per cent of wet weight; samples and symbols as in Fig. 2.

found by Love and Ironside (1958) on thawed samples of frozen fish. The decrease of extractability in these refrozen samples at −18 C is more rapid than observed in once-frozen packaged fillets stored at the same temperature by Dyer et al. (1957a, b) (Fig. 4). The rate is similar to that observed by Love (1962) on once frozen cod but "denaturation" does not proceed as rapidly as observed by this author in a previous experiment in 1958; however, in both these experiments, determinations were made on thawed samples. The only comparative data on refrozen fillets of cod are: (*a*) trap fish samples after thawing and refreezing which show rates similar to those presently found (Dyer et al., 1962); and (*b*) Grand Bank cod frozen at sea in October, 1964 and later thawed, refrozen, and stored (MacCallum et al., 1966) and which had rather poor storage life; the once- and twice-frozen fillets showed similar rates of denaturation at −23 C.

Comparison of average EPN values for all test samples with average taste-panel scores for all test samples during the first 8 months shows a coefficient of correlation of +0.924, which is significant at the 5% level using the method of Kramer and Twigg (1962). But there is no significant correlation between taste-panel scores and EPN values of the control samples, though there is no change in either over the storage period.

In summary, the chemical studies showed that storage quality of refrozen trawler-caught cod was not affected by freezing method, plate vs. brine, stage of rigor, pre- vs. post-rigor, or by method of thawing, water vs. microwave.

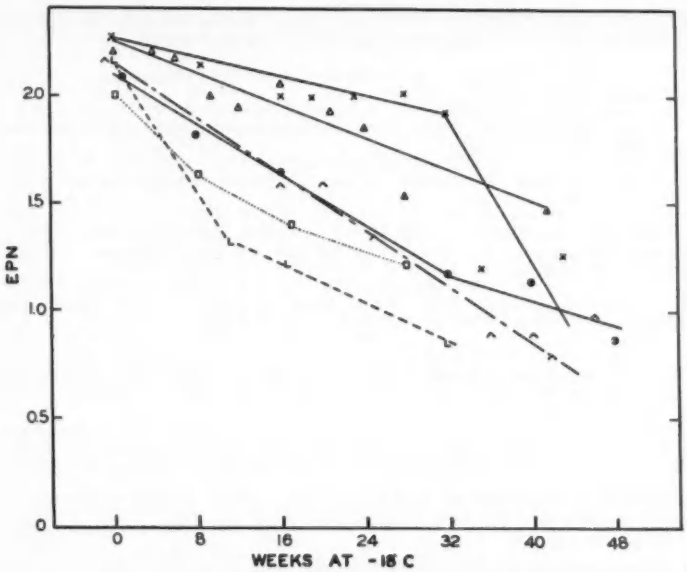


FIG. 4. Comparison of extractable protein nitrogen change in cod fillets stored at −18 C by various authors. × — cod fillets — Dyer et al., 1957a; △ — cod fillets — Dyer et al., 1957b; ● — cod fillets, refrozen — present paper; L — cod fillets, thawed prior to analysis — Love, 1958 (−18.6 C); ▲ — cod fillets, thawed prior to analysis — Love, 1962 (−20 C); □ — trap cod fillets, refrozen — Dyer et al., 1962.

The process of thawing and refreezing had little immediate effect, there being little difference between the nonfrozen and round-frozen fish. However, a very rapid development of FFA occurred in the refrozen fish in the period between 1 week and 2 months of frozen storage, and this correlated with a considerable drop in taste-panel scores in the first month of refrozen storage at −18 C.

ACKNOWLEDGMENTS

The authors are pleased to thank Mr D. G. Ellis of the Halifax Laboratory, Fisheries Research Board of Canada, Halifax, N.S., for his technical advice on microwave thawing equipment, and the Raytheon Company, Waltham, Mass. for the use of its microwave unit.

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APPENDIX I

Engineering Considerations

Microwaves

Preliminary experiments on blocks of whiting (Merluccius bilinearis).— The 3.2-kw microwave unit used in these studies consists essentially of a microwave heating chamber with a fiberglass conveyor belt passing through it. Four magnetrons mounted above the heating chamber feed microwave energy at 2450 Mcycle/sec through wave guides into an effective heating space of about 12 × 12 × 2 inches enclosed by a rectangular plastic sleeve through which the belt passes. The sleeve, which can be removed for cleaning, seals all electrical equipment from contact with the fish. Attenuation zones at the entrance and exit ports prevent microwave energy from leaking out of the chamber, and attenuation zones just under the conveyor belt in the heating chamber absorb the microwave energy when there is no load on the belt. The power supply for the magnetrons is in the base of the unit; electrical requirements are 240 v, 100 amp, 60 cycles, 3 wire, single phase. Although designed to operate at 240 v, the equipment performed satisfactorily from the 208-v supply available in the Gloucester Technological Laboratory. Exposure of the frozen fish to microwave energy can be varied by changing (1) the speed of the conveyor belt; and (2) the number of magnetrons activated.

The first tests on thawing fish in the microwave unit were made using 13½-lb blocks of headed and gutted whiting measuring 20⅛ × 11⅝ × 1½ inches.

Belt speed was held constant at selected settings to give radiation exposure times for the blocks varying from 0 to about 210 sec. The latter exposure was obtained by seven separate 30-sec exposures.

With less than 4 magnetrons in use, the distribution of microwave energy in the chamber was uneven and localized overheating resulted.

The most uniform thawing was obtained at highest belt speed (20 ft per min), which exposed the fish to the radiation for 3 sec per pass. However, since 20 or more passes through the machine were needed to thaw the fish to an internal temperature of −1.5 C (at which it was about ready for filleting), a compromise treatment that it was hoped would be feasible for the main tests was investigated. In this treatment the belt speed was varied from slow initially to fast as thawing proceeded and the blocks were reversed at each pass. Results were found to be acceptable.

Preliminary experiments on blocks of cod. — Experimental cod blocks sawed to a thickness of 1½ inches and of the same surface area as the whiting blocks were thawed using various combinations of belt speeds ranging from one pass at 60 sec plus six passes at 30 sec to four passes at 60 sec plus two passes at 30 sec.

Results best suited to handling, and at the same time producing thawing results acceptable for the main tests, were obtained using four passes at 60 sec exposure each followed by one pass at 30 sec. Nevertheless, using this technique, areas without skin were sometimes found to heat rapidly and were in danger of cooking, and some ice pockets (between fish) remained. It was apparent that in the main tests thawed samples would have to be examined carefully and any overheated fish discarded.

Thawing in polyethylene trays under water did not improve the results. The water on top of the fish heated rapidly, cooking the fish while the bottom of the block remained frozen.

Plate-frozen test fish. — Typical temperature readings of the flesh of the intact blocks of cod after five passes were: center = −1.5 C; surface at ends = 27–32 C; surface 1 inch in from ends = 13–20 C; and surface 2 inches in from ends = 13–15 C. Occasional cooking occurred at the front and rear edges of the blocks, but this could be greatly reduced by having a continuous stream of uniform blocks, separated by no more than 1 inch, on the belt.

Other observations were that:

(1) Whereas the film of moisture on the surface of the block refroze while it was being transferred to the loading end of the machine after the first pass, during subsequent transfers the moisture did not refreeze, and this was followed by very rapid thawing of the surface in contrast to the slow thawing in the center. This is due to the great difference in the absorption of microwave energy in water and ice. An extreme example of this effect was that even after four or

five passes through the microwave unit slivers of ice frozen in depressions between the fish remained intact, whereas the adjacent skin and flesh were thawed completely.

(2) Overheating can be minimized by partially thawing the fish and putting them in "buffer" storage in ice or water for several hours. This requires removal of the fish just prior to complete thawing so that equilibration in ice results in completion of thawing.

(3) The flesh of the fish appears to absorb the microwave energy much more rapidly than does the skin.

(4) More uniform thawing was found when block temperatures were above −18 C and uniformity of thawing appeared to increase with further increases above −18 C.

Brine-frozen test fish. — In general, the individual fish did not thaw satisfactorily even when great pains were taken to pack the fish close together on the belt and to keep a continuous stream flowing under the magnetrons. Severe overheating and cooking of the thinner parts of the fish occurred, so after each pass the smaller pieces had to be culled. It was also found that cooked spots about 2 inches^2 in area appeared just under the skin of these fish, and the location had no apparent relation to thickness of the fish.

General. — Estimates of operating costs for microwave equipment such as that used in these tests average about $0.15 per kilowatt hour (kwhr) of microwave power produced. Of this cost, $0.125 per kwhr is for microwave tube replacement and $0.025 per kwhr for the electrical power consumed (based on an average cost of $0.01 per kwhr for industrial power). Therefore, at the rated output of 3.2 kw, and the maximum rate of 96 lb per hour found in our tests, the cost of thawing fish will be:

$$\frac{3.2 \times 0.15 \times 1}{96} = \$0.005 \text{ per lb.}$$

Calculations of operating costs are, however, based on a 40% conversion of electrical power input to microwave power output. Our measurements showed that under full load the equipment required 81 amp from a 208-v line, or 17 kw to produce the rated output of 3.2 kw for an efficiency of only 19%. Using the 19% efficiency figure, the cost of electricity consumed per kwhr of microwave power produced will be about doubled, giving an operating cost of $0.175 per kwhr of microwave power. This will then increase the cost of microwave thawing to $0.0058 per lb, which in itself might not be prohibitive for thawing some types of packs.

The study thus showed that this microwave machine or any counterpart not fitted with appropriate on–off control of energy from the magnetrons to the cavity cannot be recommended for thawing block-frozen cod commercially. It remains to be seen whether, with proper control of energy, a cavity source would be suitable for thawing blocks of commercial thickness (of the order of 4 inches) on a commercial basis. Bengtsson's work (1964), conducted subsequently, would indicate however that blocks of up to 4 cm in thickness only can be successfully thawed in this manner.

Water-Thawing

Costs of operation (less labour costs) and efficiency of thawing

Plate-frozen fish. — To thaw 1 lb of fish (initial temperature −23 C, finishing temperature −0.5 C) requires about 125 Btu (0.037 kwhr), thus with electrical power at $0.01 per kwhr, the electrical cost (theoretical) would be $0.00037 per lb. The actual cost of electrical power including the cost of heating water to 18 C in readiness for thawing in a large commercial water thawer in Newfoundland has since been assessed at twice the above figure or $0.00074 per lb (assuming electrical power is available at $0.015 per kwhr) by personnel of the Fisheries Research Board of Canada, according to a Bulletin now in preparation. In the present tests some free heat was added to the fish through the walls of the tank and the only electrical power used was converted into heat energy by an electrically driven pump used to recirculate the water used for thawing. If operated at full load, an unlikely situation in view of the amount (50 U.S. gal per min) of water recirculated, the motorized pump would have supplied about half the heat needed to thaw 120 lb of fish in 3 hr at a maximum cost of $0.0002 per lb.

Very generous estimates of water costs in the present tests can be made by assuming 30 ft^3 to fill the tank initially, another 20 ft^3 added during thawing to maintain the temperature at 7 C, and water costs at \$4.00 per 1000 $feet^3$. The water costs are thus \$0.0017 per lb of fish thawed and total costs of water and power are not over \$0.002 per lb of fish thawed. This compares with \$0.00076 per lb for the large-scale commercial operations conducted in Newfoundland where water charges at \$0.10 per 1000 Imp gal and conservative use of water (at a rate of 0.2 gal per lb of thawed fish vs. 2.6 gal employed per lb of fish in the tests described in the present paper) were almost negligible, namely \$0.00002 per lb of fish thawed. Thus, the total cost of power and water of either \$0.00076 per lb of fish (as in commercial practice) or \$0.002 per lb of fish (as in the laboratory tests) are significantly less than the cost of \$0.0058 per lb of fish thawed in the microwave equipment.

General. — As noted earlier, after thawing in microwaves the fish rated considerably higher in appearance and texture, and slightly higher in odor, than did those thawed in water. The fillets from water-thawed fish showed a brown discoloration, particularly on the skin side, which was absent from the fillets from fish thawed in microwaves. However, in the refrozen test samples average scores showed no significant difference attributable to the thawing method. The latter conclusion was reached also by MacCallum et al. (1967) in studies on July-caught trap cod and on Grand Bank redfish, each of which were thawed at 15.5 C in recirculated tap water and in microwaves (38 Mcycles/sec) samples for a second freezing. The latter authors and the authors of the present paper are in agreement too that greater yields would be expected from water-thawed samples than from those in micro- or macrowaves due to (1) losses arising from overheating using the electrical energy methods and (2) losses either in weight or in extra time involved in filleting fish that may not be completely thawed. The latter losses may occur when the fish are removed early from the micro- and macrowave thawers to avoid overheating those individuals located on the surface and edges of the blocks.

Some Chondracanthidae (Copepoda) from Fishes of British Columbia[1]

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ABSTRACT

This paper redescribes three species of Chondracanthidae (Copepoda) parasitic on the fishes of British Columbia and previously recorded by earlier writers (*Chondracanthus irregularis*, *Ch. pinguis*, and *Ch. gracilis*). Two new species are described (*Ch. pusillus* and *Acanthochondria holocephalarum*). Finer structural details of the chondracanthid appendages are illustrated and the taxonomic status of the previously known species is discussed.

Only six species of parasitic Copepoda belonging to the family Chondracanthidae have been recorded, so far, from the waters of British Columbia. The first two were described by Wilson (1912), and Fraser (1920) added four more. The descriptions produced by both authors were barely sufficient for the identification of their newly discovered species. It appears that the literature contains no subsequent reports on these species.

The present author has had the opportunity of examining in some detail a small collection of Chondracanthidae from fishes of British Columbia. Some specimens came from the collection of Dr H. P. Arai, University of Calgary, Calgary, Alta.; others had been kept for some time in the collections of the Biological Station, Fisheries Research Board of Canada, Nanaimo, B.C. One species was collected by Dr Gordon Bell.

It is the aim of this paper to redescribe some of the species previously recorded and to describe two which the author believes to be new to science.

Those workers who investigated the chondracanthid copepods in the past were faced with an onerous task. With the possible exception of the thoracic legs, the morphology of the appendages within the family (and particularly within the subfamily Chondracanthinae) is monotonously uniform and affords very few distinguishing characters. Consequently, the members of this subfamily are classified mainly by the arrangement, shape, and size of their cephalothoracic and trunk outgrowths. These outgrowths form a profuse variety of combinations and are fairly useful for the purposes of classification. Unfortunately, however, their structure is not always rigidly constant within the limits of individual species. It must be taken into account that the age, the stage of maturity, and the infraspecific variations can be reflected in the parasite's appearance, just as much as can influence of the host association (in parasites which are known to have more than one host species). Even

[1]Received for publication November 1, 1967.

J. Fish. Res. Bd. Canada, 25(2): 321–345, 1968.
Printed in Canada.

where clear evidence of distinctness is demonstrable, there is as yet no general agreement whether the differences observed can be accepted as merely specific, or whether they have significance also at the generic level. For example, the only difference between the genera *Acanthochondria* and *Acanthochondrites* is the structure of their thoracic legs which are biramous in the former and uniramous in the latter. On the other hand, the present genera *Chondracanthus* and *Chondracanthopsis* contain species with bipartite and tripartite legs.

The author's attention, therefore, turned to the finer structural features of the appendages in the hope that some details might be discovered which would be helpful in the identification of species. To avoid the tedium of re-describing the limbs of each species in full, a brief summary of the structure of the chondracanthid limbs is given below. The features listed are common to all species of Chondracanthinae known to the author.

(*1*) *First antenna.*—Uniramous, cylindrical, usually tapering towards the tip, not distinctly segmented, with or without apical armature and setae along its anterior margin.

(*2*) *Second antenna.*—Uniramous in the female in which the endopod is modified into a strong hamulus. In the male of some species a reduced exopod is also present.

(*3*) *Mandible.*—Consists of two parts. Basal part squat, angular, with broad base. Distal part falcate, denticulated along two margins. Tips of opposing mandibles meet in the centre in front of the buccal orifice.

(*4*) *Mandibular palp.*—Small and very difficult to study. Its homology not well understood. Heegaard (1947) believes it to be first maxilla. (Most workers find this appendage too difficult to study and many illustrations of it are uninformative or even misleading. Seen in its full extept from either the ventral or dorsal surface, the mandibular palp is almost cordiform (Fig. 38), having a convex lateral margin and a concave medial margin. The distal tip is always equipped with two spines: a strong terminal and a shorter, more slender subterminal spine. In some species the proximal part of the medial margin might also carry a spine or produce a pointed projection.)

(*5*) *Maxilla.*—Consists of two parts. Basal part squat with broad base. Distal part forms a claw with varying numbers of denticles along one or both margins. Near the base of claw auxiliary spine of varying length (*aux*, Fig. 27) and basal seta (*bs*, Fig. 27). (As far as the author is aware, the basal seta has not previously been reported.)

(*6*) *Maxilliped.*—Consists of three parts. Basal part forms "arm" of the limb and is its most powerful and muscular component. It articulates with cylindrical central part, distally provided with two spiny pads. The spines (Fig. 42) are set closely together, about 10 μ long, sharp-pointed. Terminal part forms claw, broad at the base, tapering to a curved tip. A denticle often present on the concave margin of the claw.

(*7*) *Caudal lamina.*—Cylindrical at the base but flattened distally from side to side. Commonly equipped with two ventral and one dorsal seta, all three near the base of the lamina. Other structures might also be present.

This paper does not contain a section of general discussion. Individual discussions follow the description of each species.

The specimens of each species dealt with in this paper were examined both entire and dissected. The examination of the dissected appendages was carried out in Berlese's fluid, used as mountant and clearing agent. No stains were used but the details of the specimens were examined in phase contrast illumination and in Nomarski's interference phase. The drawings are all free-hand, made with the aid of an eyepiece graticule.

DESCRIPTION OF THE SPECIES

Chondracanthus irregularis Fraser, 1920
Syn.: *Chondracanthus dogieli* Gusev, 1951
Chondracanthopsis dogieli (Gusev, 1951) Markevich, 1956

Record of specimens: One ovigerous female, with male attached, was collected by Dr H. P. Arai in Nalau Pass (near Fitz Hugh Sound), B.C., on July 7, 1965. Host: *Myoxocephalus polyacanthocephalus* (Pallas, 1811). Habitat: Gill cavity.

DESCRIPTION OF THE FEMALE

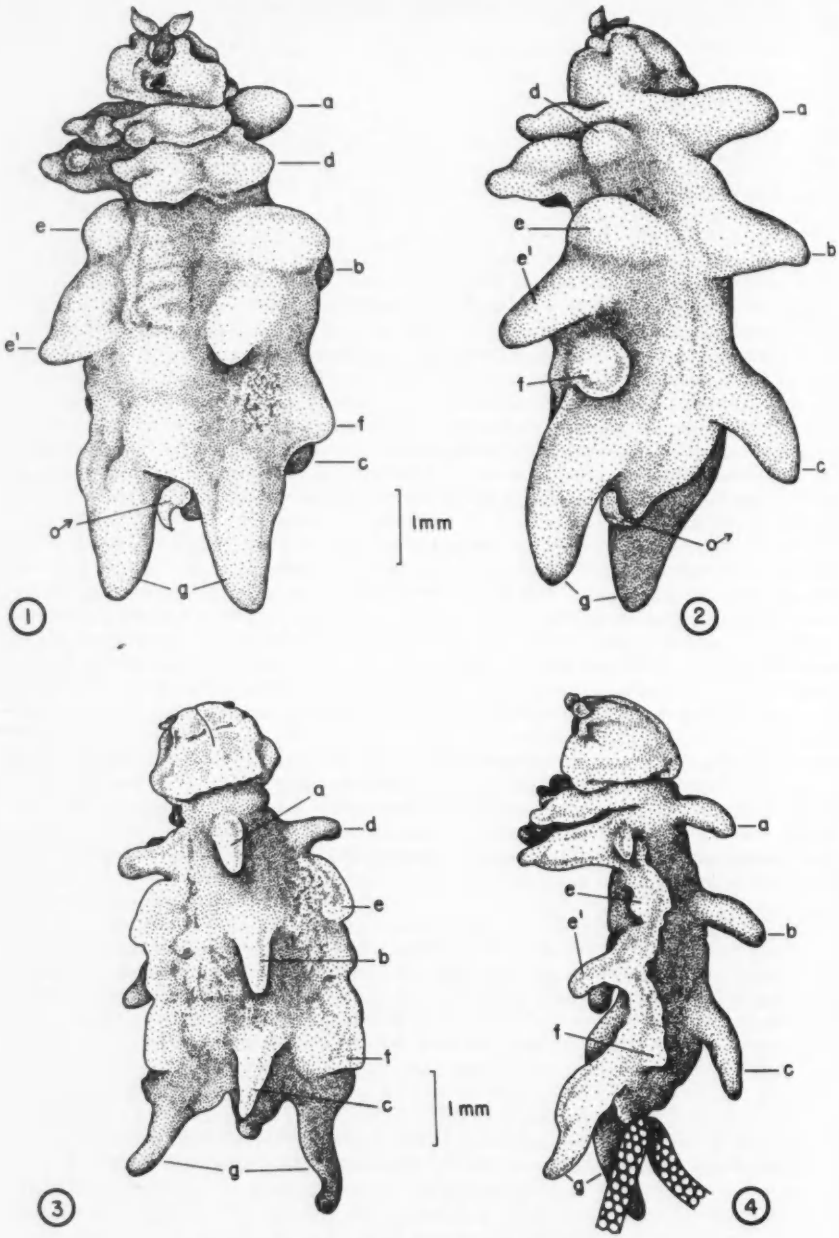
General appearance (Fig. 1–4). Cephalothorax wider than long, with fairly prominent and inflated first antennae on its anterior margin and with angular posterolateral corners. Trunk separated from cephalothorax by neck-like constriction with obscure segmentation. On its dorsal wall are three prominent processes (*a*, *b*, and *c* in Fig. 1–4). These processes are digitiform and may or may not be wider at the base. Trunk has also three lateral processes (*d*, *e*, and *f*). The space between *d* and *f* is occupied by a large and more complicated outgrowth *e*. The latter consists of an anterior part *e*, connected with a posterior part *e'*, digitiform and pointing obliquely ventrad and backwards. In addition, the posterior extremity of the trunk is produced into yet another pair of processes *g*, which are a common feature in most chondracanthids of the subfamily Chondracanthinae and are often referred to as the posterior processes. Between the processse rests the genital–abdominal tubercle which is tipped with the caudal furca and to which the male is usually attached.

The appendages of *Ch. irregularis* were amply described by Gusev (1951). This description is concerned only with some amendments of the previous descriptions.

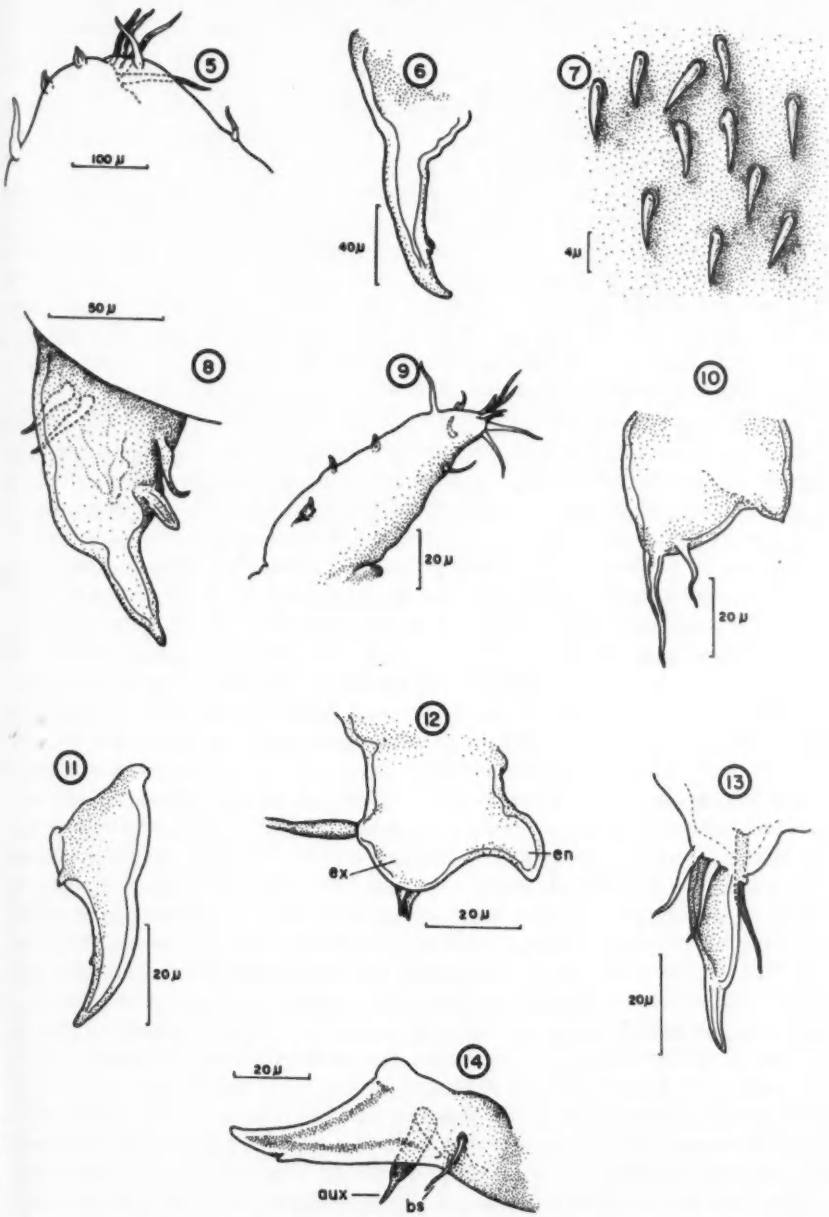
The first antenna has been described as having four apical setae. As shown in Fig. 4 there are at least seven setae in apical and two in subapical positions. The second antenna is not distinguished by any diagnostic features. This is also true of the mandible, mandibular palp, and maxilla, all of which have been illustrated by Gusev (1951). The only detail missing from Gusev's description is the basal seta of the maxilla present in both sexes and shown for the male in Fig. 14. The female maxilliped is distinguished (Fig. 6) by the presence of two small denticles on the concave margin of the claw in place of the more usual single one. The thoracic legs, biramous and of common chondracanthid type, are covered with fine denticles (Fig. 7) which are slender, about 5 μ long, distributed in numbers of 10–12 per area of 400 μ^2 (one such area is shown in Fig 7). The caudal furca (Fig. 8) has not been previously described in detail. As mentioned in the introduction, this is a fairly complicated structure. In addition to the three setae mentioned previously, each lamina is also equipped with a robust conical protuberance seen in Fig. 8 on the margin just below the dorsal seta.

DESCRIPTION OF THE MALE

There is nothing distinctive about the general appearance of the male. It is quite similar to other males of *Chondracanthus* and has been drawn by Gusev (1951). Some details of its appendages, however, have not been clearly shown. The first antenna has the apical armament consisting of eight setae (Fig. 9), only seven of which can be seen from the aspect shown. Its anterior margin also carries at least five other structures, the most distal of which consists of a longer seta and a short, conical protuberance. The mandibular palp (Fig. 10) is very small with two terminal spines long and slender and with a suggestion of a minute prickle on the proximal convexity of the medial margin. There is one denticle on the maxillary claw (Fig. 14) and one on the claw of the maxilliped (Fig. 11). In some specimens a small tubercle was also observed near the base of the claw of the latter. The two pairs of thoracic legs are extremely difficult to study and have not been hitherto shown in detail. Both pairs appear to have the same structure (Fig. 12). They have a distinguishable exopod (*ex*) and an endopod (*en*) which is very prominent and has a beak-like curve. The endopod is unarmed but the rounded tip of the exopod carries three short spinules. A long seta, longer than the width of the entire limb, is also present on the lateral margin of the leg, marking the base of the exopod. The caudal laminae (Fig. 13) resemble those of the female but have relatively a much smaller conical protuberance on their medial margins.



FIGURES 1–4. ***Chondracanthus irregularis***, female.
Fig. 1: Canadian specimen, entire, ventrolateral; Fig. 2: The same specimen, dorsolateral; Fig. 3: Russian specimen, entire, dorsal; Fig. 4: The same specimen, lateral. For lettering, see text.



FIGURES 5–14. ***Chondracanthus irregularis***, female and male.
Fig. 5: Female, tip of first antenna; Fig. 6: Claw of maxilliped; Fig. 7: Spinulation of second thoracopod, detail; Fig. 8: Caudal lamina; Fig. 9: Male, first antenna entire, dorsal; Fig. 10: Mandibular palp, ventral; Fig. 11: Claw of maxilliped; Fig. 12: Second thoracopod, ventral; Fig. 13: Caudal lamina, ventral; Fig. 14: Claw of maxilla, ventral. *aux* — auxiliary spine; *bs* — basal seta; *en* — endopod; *ex* — exopod.

Discussion

In describing *Ch. dogieli*, Gusev (1951) based his diagnosis on the differences between his specimens and the original description of *Ch. irregularis*. Acknowledging the existence of the general resemblance between them, he found these differences sufficient to place his specimens in a new taxon. The following differences were taken into account: (1) the dorsolateral processes of the trunk described by Fraser (1920) appeared to be absent from Gusev's specimens; (2) the anterior dorsal outgrowth was stated by Fraser to be situated on the second thoracic segment whereas Gusev believed that in his specimens it was on the third segment; (3) what was termed the "ventral outgrowth" of the fourth segment appeared to be of different shape in the two copepods; (4) Fraser's male was shown to have a clear demarcation line between the genital segment and the abdomen but no such line was observed by Gusev; (5) a number of other small differences which were not specified by Gusev.

In view of the obvious resemblance between *Ch. irregularis* and *Ch. dogieli*, a detailed comparison of these two species was necessary. Fraser's type specimens, as far as the author could ascertain, are no longer in existence. The comparison, therefore, was made between the specimen collected in British Columbia (and closely resembling Fraser's original description) and three specimens from Gusev's collection of *Ch. dogieli*. Special attention was given to the points of difference mentioned in the description of the latter parasite.

As stated earlier, the number, shape, and arrangement of the trunk and cephalothoracic processes are diagnostically significant in Chondracanthinae. The comparisons between Fig. 1 and 2, on the one hand, and Fig. 3 and 4, on the other, show clearly that there are no differences between the number and the arrangement of these processes, labelled in the drawings *a* to *g*. There are, however, some small differences in their shapes. The trunk of the Canadian specimen is stouter and more robust than that of the Russian specimens; similarly, its trunk processes are generally thicker, especially at their bases. The first dorsal process (*a*) arises immediately behind the posterior margin of the carapace in the Canadian specimen (Fig. 2), whereas in the Russian ones a small gap is present between that margin and the base of the process (Fig. 4). This fact can be attributed to the generally more slender shape of the processes in the Russian parasite. The segmentation of the chondracanthid trunk is usually quite obliterated externally. In this instance there is certainly no ground for assigning the position of the process *a* to the second or third segment. A more definite difference of shape exists between processes *d* which are relatively longer in the Russian parasite than in the Canadian one. Some differences can be seen between processes *e*, particularly in the relationship between *e* proper and *e'*, in both parasites. This outgrowth, referred to as the "ventral outgrowth," was one of the reasons for the erection of *Ch. dogieli*, but Fig. 1–4 make it clear that the difference is, in fact, slight enough to be attributed to infraspecific variation. It must also be taken into account that the Russian specimens showed some evidence of being affected by the

conditions of storage and preservation, their trunks bearing traces of shrinking. This in itself could have resulted in the differences mentioned by Gusev.

As regards the male, the solid line of demarcation between the genital segment and the abdomen was due merely to the techniques of illustration used by Fraser and does not exist as a morphological feature.

There were no differences between the structural details of the appendages of the two females. In the male these differences were restricted to the maxillary claw; the claw of the Russian specimens carried from one (as shown in Fig. 14) to four denticles on its margin, whereas in the only Canadian male no denticles were observed. It should be pointed out that the structure of the thoracic legs of both males appeared quite similar. There are reasons to believe that the structure of these appendages might be useful from the taxonomic point of view.

The author concludes that *Ch. dogieli* does not differ sufficiently from *Ch. irregularis* to be accorded the status of an independent species and should be considered as synonymous with it. Further study of the species might reveal enough intraspecific distinctions to allow a separate infraspecific taxon for the Russian parasite to be considered but this stage has not yet been reached.

As recorded in literature, *Ch. dogieli* parasitizes fishes belonging to the genus *Myoxocephalus*. Gusev (1951) recorded it from *M. stelleri* and *M. stelleri decastriensis*; Markevich (1956) found it on an unspecified member of this genus in the Bering Sea, and Kabata and Gusev (1966) reported it from *M. batrachoides* and *M. brandti*. The Canadian specimen described in this paper came from *M. polyacanthocephalus*. Fraser's (1920) type specimen was found on *Enophrys bison*, a fish belonging to a genus of sculpins closely related to *Myoxocephalus*. Thus, the host affinities also suggest that these two parasites might be identical.

Another important point, raised by the study of *Ch. irregularis*, is the validity of the genus *Chondracanthopsis*, to which Gusev's species was transferred by Markevich (1956). The genus was suggested by Wilson (1932) to accommodate *Ch. nodosus* (Müller, 1779). By a coincidence of publication timing the description of the genus was first published by Oakley (1930), who obtained it from and acknowledged it to Wilson, but who was credited with the genus by some later writers (Yamaguti, 1963).

Comparing the diagnosis of *Chondracanthus* with that of *Chondracanthopsis*, as given by Wilson (1932), one finds the following reasons given for the separation of the latter: (1) the cephalothorax of *Chondracanthopsis* is much wider than long, in contrast to *Chondracanthus*; (2) the trunk of *Chondracanthopsis* has the dorsum provided with a row of rounded laminae, semilunar and folded back against the dorsal surface; in *Chondracanthus* the outgrowths are digitiform; (3) the thoracic legs of *Chondracanthopsis* are uniramous with a winged basal part and a boot-shaped distal part; in contrast, the legs of *Chondracanthus* are biramous.

Subsequent authors added only little to the diagnoses of the two genera. According to Markevich (1956), *Chondracanthopsis* has a cephalothorax devoid

of posterolateral outgrowths, whereas *Chondracanthus* is equipped with these processes. On the basis of this feature he transferred Gusev's *Ch. dogieli* to *Chondracanthopsis* even though its dorsal processes are distinctly digitiform and described *Chondracanthopsis multituberculata*, also with digitiform dorsal processes. Yamaguti (1963) amalgamated the earlier diagnoses without adding any new characters.

It is rather curious that no writer involved in these decisions appears to have made any reference to the type species of the genus *Chondracanthus* in drawing conclusions as to the affinities of the species in which they were interested. Had such references been made, a lot of confusion could have been avoided.

It seems that Markevich's (1956) view about the presence or absence of the posterolateral outgrowths on the cephalothorax might be acceptable under some conditions as a discriminant at the generic level. For example, one of the diagnostic features of *Lateracanthus* is the presence of these processes. The uniramous structure of the thoracic legs has been used to discriminate between the genus *Acanthochondrites*, which has such legs, and *Acanthochondria*, whose legs are biramous. These two features, therefore, might be considered as useful at the generic level of discrimination. However, the type species of the genus *Chondracanthus*, *Ch. zei* De La Roche, 1811, has no posterolateral cephalothoracic outgrowths. These outgrowths are also absent from the type species of *Chondracanthopsis*, *Ch. nodosus* (Müller, 1779). As regards the structure of the thoracic legs, both these species have legs described as uniramous with a boot-shaped distal part. The legs of *Ch. nodosus* are shown in Fig. 72 and 73 and those of *Ch. zei* in Fig. 74 and 75. These figures make it quite plain that no generic discrimination is possible on the ground of the leg structure of these two species. (Comparing Fig. 72–75 with, e.g., Fig. 63, one wonders why one of these legs should be considered as uniramous, whereas the other is believed to be biramous. The author is convinced that the boot-shaped part of the leg of the species discussed is merely a lobe, developed from one ramus of a biramous leg.)

The only feature left as a suggested discriminant between the two genera is the dorsal outgrowths of the trunk. It has been suggested earlier in this paper that such differences are sufficiently accounted for at the specific level of classification. There are, therefore, no grounds for discrimination between *Chondracanthus* and *Chondracanthopsis*. The author proposes that *Chondracanthopsis* be suppressed and that all species at present included in it be placed in the genus *Chondracanthus*.

There remains to be taken into account the awkward fact that of all the species of *Chondracanthus* known at the present time, there are only two with the peculiar tripartite leg structure. The question arises whether this calls for the general revision of the genus, or whether it should only be taken as reflecting on the diagnostic value of the legs. If the latter is accepted, more attention should be paid to the genus *Acanthochondrites* and its relationship with *Acanthochondria*. These problems, however, must remain outside the present paper.

Chondracanthus pinguis Wilson, 1912
Syn.: *Chondracanthus slastnicovi* Gusev, 1951

Record of specimens: Several ovigerous females, with males attached, were collected 42 miles S × W of Amphitrite Point, west coast of Vancouver Island, B.C., by the vessel *Sharlewis K*, on Feb. 13, 1967. Host: *Sebastodes alutus* (Gilbert 1890). Habitat: Gill cavity.

Description of the Female

General appearance (Fig. 15 and 16). Cephalothorax wider than long, its posterolateral corners produced into long, digitiform processes which stretch far backwards and slightly curve in a medial direction. First antennae prominent and inflated. Sharp groove between cephalothorax and trunk. The latter has four conical swellings in the middorsal line and two lateral processes on each margin. These processes extend and curve in a manner similar to that of the cephalothoracic ones. There are also two posterior processes of the trunk; in some specimens these tend to curve inwards so as to overlap in midline.

The tip of the first antenna (Fig. 17) has an armament of eight apical setae. Neither the second antenna nor the mandible has any distinguishing features. The mandibular palp (Fig.18) resembles that of the preceding species. The dorsal margin of the maxillary claw (Fig. 19) is armed with 14 denticles and the auxiliary spine is smooth and tapers abruptly to a fine point. The claw in Fig. 19 has been shown in posterior view, to display the marginal denticles more prominently. The tip of the claw was bent in the course of handling and appears hooked, which is an artifact. There is one denticle on the concave margin of the claw of the maxilliped (Fig. 20). There appear to be no denticles on the cuticle of the thoracic legs. On their lateral margins, at the base of the exopod, there is a short seta (Fig. 21), encountered by the author in the majority of Chondracanthinae. The tip of the exopod (Fig. 21, inset) is provided with three elongated, soft papillae, presumably sensory. The caudal laminae differ from those of the preceding species in having no conical outgrowth on the medial margins. The three setae are present (Fig. 22).

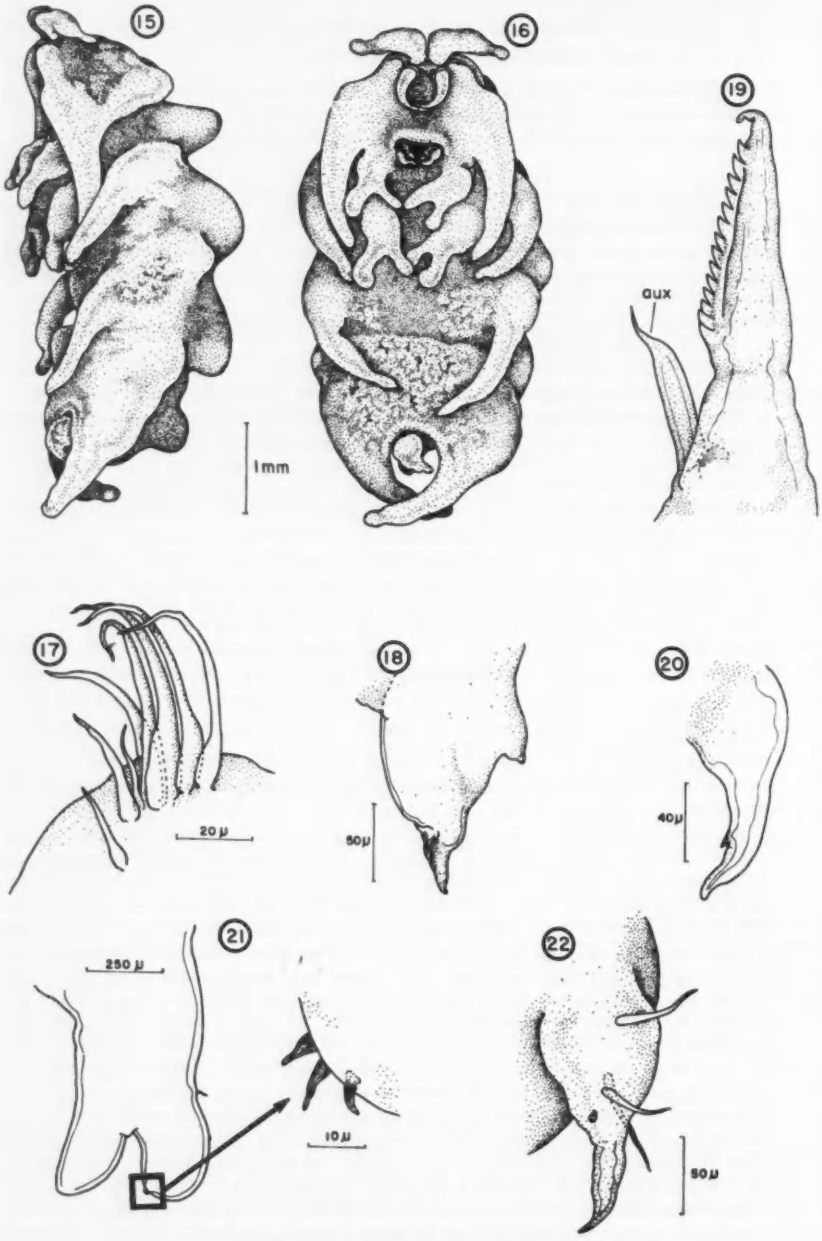
Based on four specimens, the dimensions (in mm) of this species are: total length — 3.62–4.90; cephalothorax length — 0.93–1.06; cephalothorax width — 1.70–1.91; trunk width — 1.91–2.42.

Description of the Male

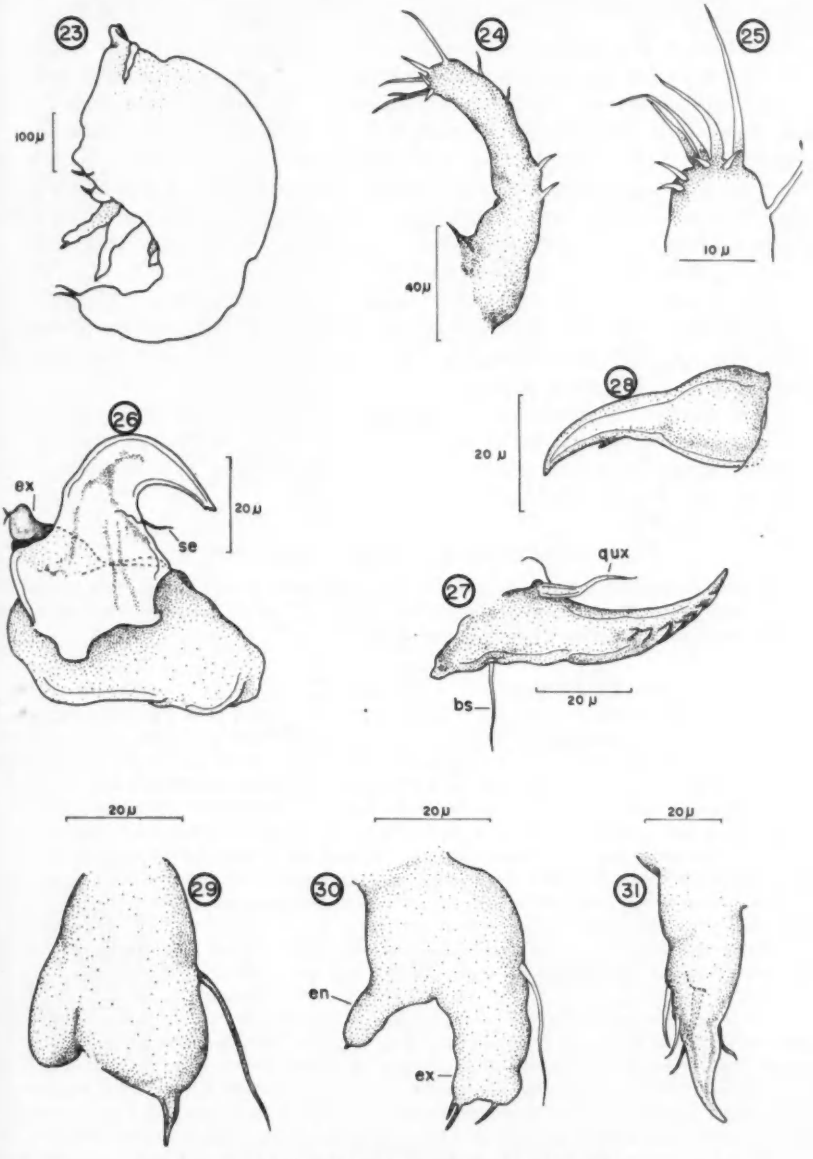
General appearance (Fig. 23) similar to those of other males of the genus. The anterior, almost circular, part (cephalothorax) is followed by a narrow and cylindrical part, indistinctly segmented and curving ventrally.

The first antenna (Fig. 24 and 25) is short and slender, with a well-developed apical armament (Fig. 25) consisting of eight setae. The anterior margin carries an additional armament (Fig. 24). The second antenna (Fig. 26) retains its exopod, in addition to the hamate endopod which is usually present. On the inner margin of the latter there is a fine seta (*se*, Fig. 26). The exopod is short and cylindrical and appears to be tipped by two short setae. In Fig. 26 it is shown bending towards the observer from the ventral side of the limb, so as to appear foreshortened. The mandible is a scaled-down version of the female appendage, remarkable only because of the disparity in the size of the dorsal and ventral marginal denticles. The former are smaller and fewer than the latter. The terminal claw of the maxilla (Fig. 27) carries dorsally seven denticles. Its auxiliary spine is long and slender, as is also the basal seta. The claw of the maxilliped (Fig. 28) has the usual single denticle on its concave margin. The two pairs of thoracic legs differ in structure. The first leg (Fig. 29) is roughly oval with a well-developed, though short, rounded lobe of the endopod. The exopod, broad and round, ends in a single, sturdy spine. The lateral margin is equipped with a long, flagellate seta. The second leg (Fig. 30) is deeply cleft between the rami. The endopod is long and narrow with a rounded tip and a tiny denticle, at the apex; the exopod is broader, somewhat truncated apically and equipped with three short setae. As in the first leg, a long flagellate seta is present on the lateral margin. The caudal laminae (Fig. 31) appear to possess four setae each, rather than the three occurring in the female. An additional short and slender seta nearer to the tip of the lamina was observed, with difficulty, by the author.

Measured in situ the male is about 0.6 mm long.



FIGURES 15–22. ***Chondracanthus pinguis,*** female.
Fig. 15: Entire, lateral; Fig. 16: Entire, ventral; Fig. 17: Tip of first antenna; Fig. 18: Mandibular palp, dorsal; Fig. 19: Claw of maxilla, posterior; Fig. 20: Claw of maxilliped; Fig. 21: First thoracopod, with inset showing details of sensory papillae; Fig. 22: Caudal lamina, ventral. *aux* — auxiliary spine.



FIGURES 23–31. ***Chondracanthus pinguis,*** male.
Fig. 23: Entire, outline lateral; Fig. 24: First antenna entire, dorsal; Fig. 25: First antenna, tip, dorsolateral; Fig. 26: Second antenna, dorsal; Fig. 27: Claw of maxilla; Fig. 28: Claw of maxilliped; Fig. 29: First thoracopod; Fig. 30: Second thoracopod; Fig. 31: Caudal lamina. *aux* — auxiliary spine; *bs* — basal seta; *en* — endopod; *ex* — exopod.

Discussion

Although the original description of *Ch. pinguis* was rather scanty, it was sufficient for the purposes of identification. It is regrettable that Gusev (1951) was unaware of it, when describing his *Ch. slastnicovi*, as evident from the absence of Wilson's paper from the list of references. A comparison of Gusev's Fig. 4 with Fig. 15 and 16 of this paper is quite sufficient to convince one that the two species are synonymous. This conviction is further strengthened by the fact that both of them have been found on hosts belonging to the genus *Sebastodes* (*Ch. pinguis* on *S. auriculatus* and *S. pinniger* in addition to hosts of other genera; *Ch. slastnicovi* on *S. glaucus*).

There are two more species of the genus *Chondracanthus* whose affinities with *Ch. pinguis* should be investigated. They are *Ch. polymixiae* Yamaguti, 1939, and *Ch. theragrae* Yamaguti, 1939. The former, in particular, appears very similar to Wilson's species.

The distinctive characters of the female *Ch. pinguis* are the characteristic processes of the cephalothorax and trunk. The male is distinguished by the presence of a vestigial exopod in its second antenna and by differences in the structure of its thoracic legs.

Chondracanthus gracilis Fraser, 1920

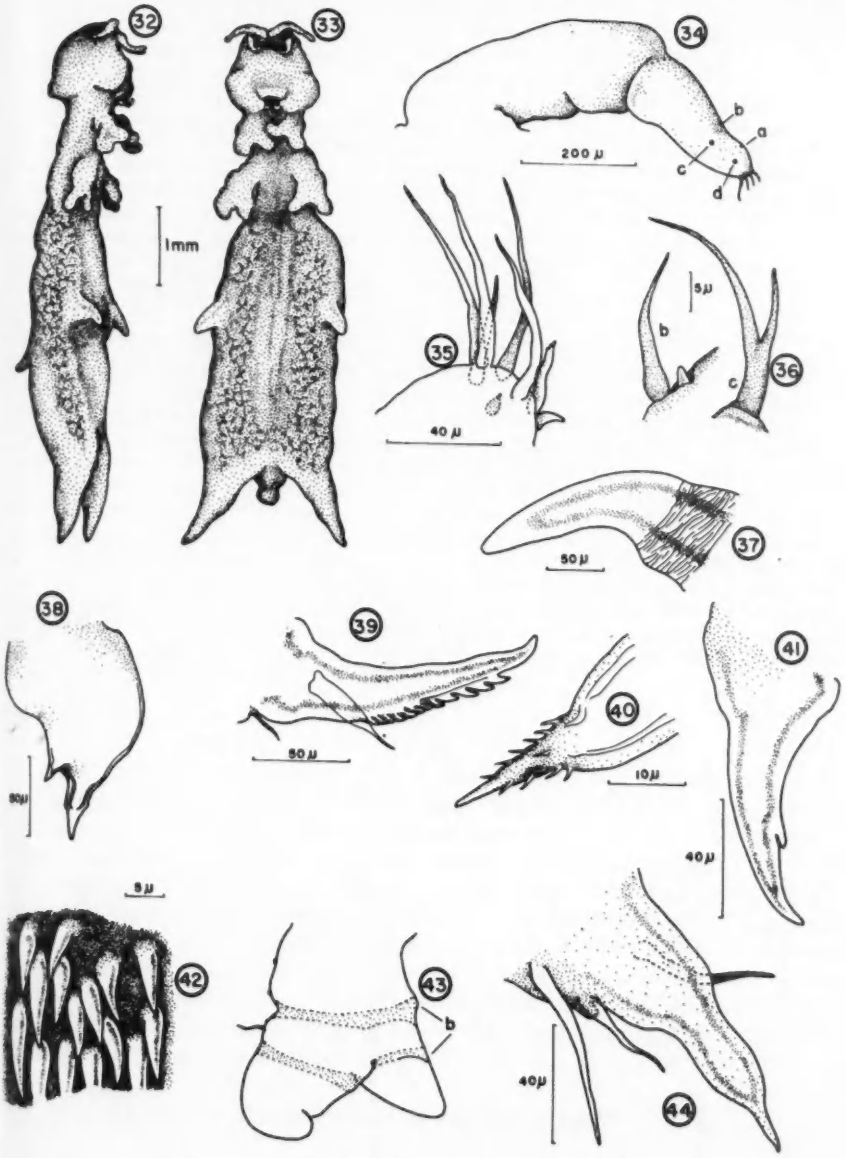
Record of specimens: Five ovigerous females, with males attached, were collected on November 29, 1961, in Departure Bay, Vancouver Island, B.C. Host: *Scorpaenichthys marmoratus* (Ayres, 1854). Habitat: Inner surface of operculum.

Description of the Female

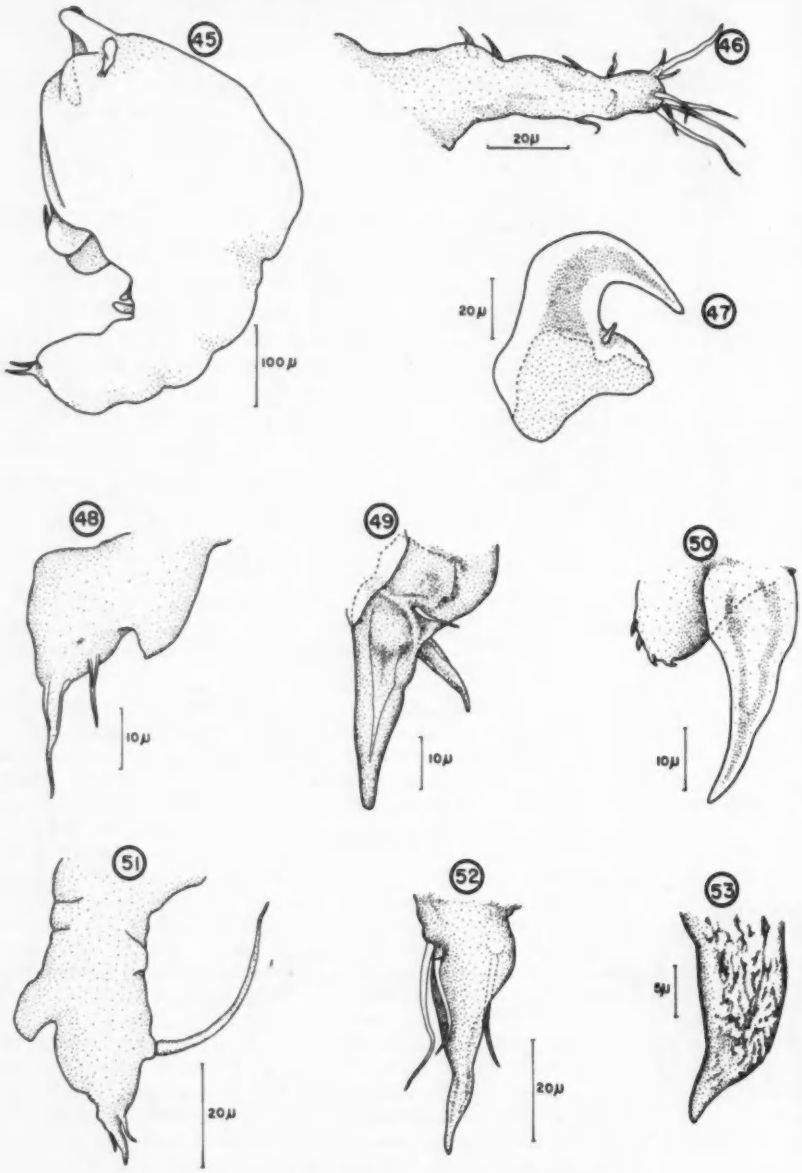
General appearance (Fig. 32 and 33). *Chondracanthus gracilis*, as the name suggests, is slender and smooth. Cephalothorax wider posteriorly, anterior part narrowing and transversely truncated. Its ventral surface forms two posterolateral swellings at the level of the buccal orifice. There is no distinct neck but the anterior part of the trunk, on which the thoracic legs are situated is much narrower than the remainder of the trunk, rapidly expanding in width beyond the level of the second pair of legs. That part of the trunk is fairly broad, dorsoventrally flattened, with almost parallel lateral margins. There is one pair of small and conical lateral processes at about midlength of the trunk. A similar single outgrowth is present in the middorsal line, just behind the level of the lateral outgrowths (Fig. 32). The posterior processes of the trunk are slightly compressed from side to side and diverge at the tips.

Based on two specimens the measurements of the female (in mm) are: total length — 7.22–8.07; cephalothorax length — 1.06; cephalothorax width — 1.06–1.34; trunk width — 1.70–1.91.

The first antenna (Fig. 34–36) is slender and fairly long, with a marked subdivision at about two-thirds of its length from the base (Fig. 34). The apical armature (Fig. 35) consists of eight setae, one of which has been shown broken off in the drawing. Near the apex of the limb there are four more structures (Fig. 36) labelled *a* to *d* in the figure. Of these *a* and *d* are simple slender setae; *b* consists of two parts placed closely together: a short cone and a longer, tapering seta. (A similar structure was mentioned in the description of *Ch. irregularis*.) The remaining seta *c* seems to have been formed by the fusion of two setae. In some specimens its position is occupied by an ordinary, single seta. The hook of the second antenna (Fig. 37) narrows abruptly near its tip, the area near the narrowing being covered by a ring of transverse striations, undoubtedly helpful in adding to the friction between the antennae and the tissue of the host. The mandibular palp (Fig. 38) is distinguished by the relatively small proximal lobe of the medial



FIGURES 32–44. ***Chondracanthus gracilis***, female.
Fig. 32: Entire, lateral; Fig. 33: Entire, ventral; Fig. 34: First antenna, entire, ventral; Fig. 35: First antenna, tip, ventral; Fig. 36: First antenna, two details of armature; Fig. 37: Second antenna, tip of hook; Fig. 38: Mandibular palp; Fig. 39: Maxillary claw (auxiliary spine ghosted); Fig. 40: Maxillary claw, tip of auxiliary spine; Fig. 41: Claw of maxilliped; Fig. 42: Detail of spinulation, maxilliped; Fig. 43: Thoracopod, diagrammatic; Fig. 44: Caudal lamina, ventral. *b* — areas of bare cuticle without spines.



FIGURES 45–53. ***Chondracanthus gracilis***, male.
Fig. 45: Entire, outline, lateral; Fig. 46: First antenna, entire, ventral; Fig. 47: Second antenna, hook; Fig. 48: Mandibular palp; Fig. 49: Claw of maxilla, posterior; Fig. 50: Claw of maxilliped; Fig. 51: First thoracopod, ventral; Fig. 52: Caudal lamina, ventral; Fig. 53: Caudal lamina, tip, lateral.

margin. The terminal spines are robust and well developed. The maxillary claw (Fig. 39) has 12 denticles along its dorsal margin. In the drawing, which shows the claw from its ventral side, the auxiliary spine is ghosted so as not to obscure the denticulation. The spine itself (Fig. 40) is characteristic in having the tip covered with slender, hair-like setules. The claw of the maxilliped (Fig. 41) has a single denticle on its concave margin. Figure 42 gives an idea as to the type and distribution of the spinules on the spinulated pads of that limb. The thoracic limbs (Fig. 43) are covered with fairly heavy denticulation similar to that occurring in *Ch. irregularis* and covering almost the entire surface of the limbs with the exception of some transverse grooves (marked *b* in Fig. 43). There are from 9 to 12 denticles in an area of 400 μ^2. In addition to the usual short seta on the lateral margin of the legs, some small papillae were also observed. The structure of the caudal laminae (Fig. 44) is of the usual type but a small, conical outgrowth is possibly also present in addition to the three setae.

Description of the Male

The general appearance (Fig. 45) of the male resembles that of the other males of the genus. As in the female, the first antenna (Fig. 46) is armed apically with eight setae and has additional armature along its anterior margin. The hook of the second antenna (Fig. 47) carries near its base on the concave margin a short peg-like structure. The mandibular palp (Fig. 48) has a fairly prominent proximal lobe of the medial margin. The maxillary claw (Fig. 49) is distinguished by the absence of marginal denticles as is the claw of the maxilliped (Fig. 50) from which the usual denticle of the concave margin is absent. The two pairs of thoracic legs have the same structure (Fig. 51). The medial lobe (endopod) is smaller than in the previously described species but it also is slightly curved. The exopod is much larger and tipped with three spinules, one of which is much larger than the other two. A flagellate seta on the lateral margin is present as in the other species. The caudal laminae (Fig. 52 and 53) are equipped with the usual three setae. As is common in these parasites, the distal part of each lamina is compressed and the lateral surfaces of these flat parts (Fig. 53) appear corrugated or sculptured. The total length of the male is in the order of 0.6 mm.

Discussion

The characteristic trunk of this species, slender and smooth, with only three outgrowths, affords a distinguishing feature allowing its identification without difficulty. With a single exception there are no species of *Chondracanthus* which can be confused with *Ch. gracilis*. The exception is *Ch. gracilis* var. *intermedia* described by Wilson (1935). Taken from *Scorpaena guttata* (a host related to that of the present species), it differs from typical *Ch. gracilis* in a number of small details, the most important of which appears to be the presence of a second dorsal outgrowth. Without making a detailed examination of Wilson's specimens, it is at present impossible to decide whether he was correct in identifying them as a variety of *Ch. gracilis* or whether they should have been placed in a separate species.

Chondracanthus pusillus sp.nov.

Record of specimens: Two females, one ovigerous and both with males attached, were collected by Dr H. P. Arai in Departure Bay, Vancouver Island, B.C., in the summer of 1963. The ovigerous specimen becomes the holotype of the species and is deposited in the National Museum in Ottawa. The other female and both males were dissected in the course of examination. Host: *Apodichthys flavidus* Girard, 1854. Habitat: Gill cavity.

Description of the Female

General appearance (Fig. 54 and 55). The most striking feature of this species is its diminutive size which distinguishes it sharply from all other local chondracanthids and which, in view of the fact that one of the specimens was ovigerous, is in all likelihood the definitive size of the species. The cephalothorax constitutes about one third of the parasite's total length. It is roughly rounded and separated from the trunk by only a slight constriction. At the anterior margin, the first antennae are conspicuously long, extending laterally beyond the limits of the cephalothorax. The trunk is short and ovoid. It has three conical outgrowths in the middorsal line. The first of these is the smallest and, situated directly behind the posterior limit of the cephalothorax, the central one is the largest. The lateral margins are also equipped with outgrowths. In the ovigerous specimen (Fig. 54) there are two such outgrowths on each margin, the first of them at the level of the first dorsal process and the second between the second and third dorsal process. In the non-ovigerous specimen the lateral processes are far less developed, the first (Fig. 56) being relatively short and the second barely recognizable as an incipient swelling. It is possible that the processes of this species develop with age. The posterolateral corners of the trunk extend into the usual posterior trunk processes.

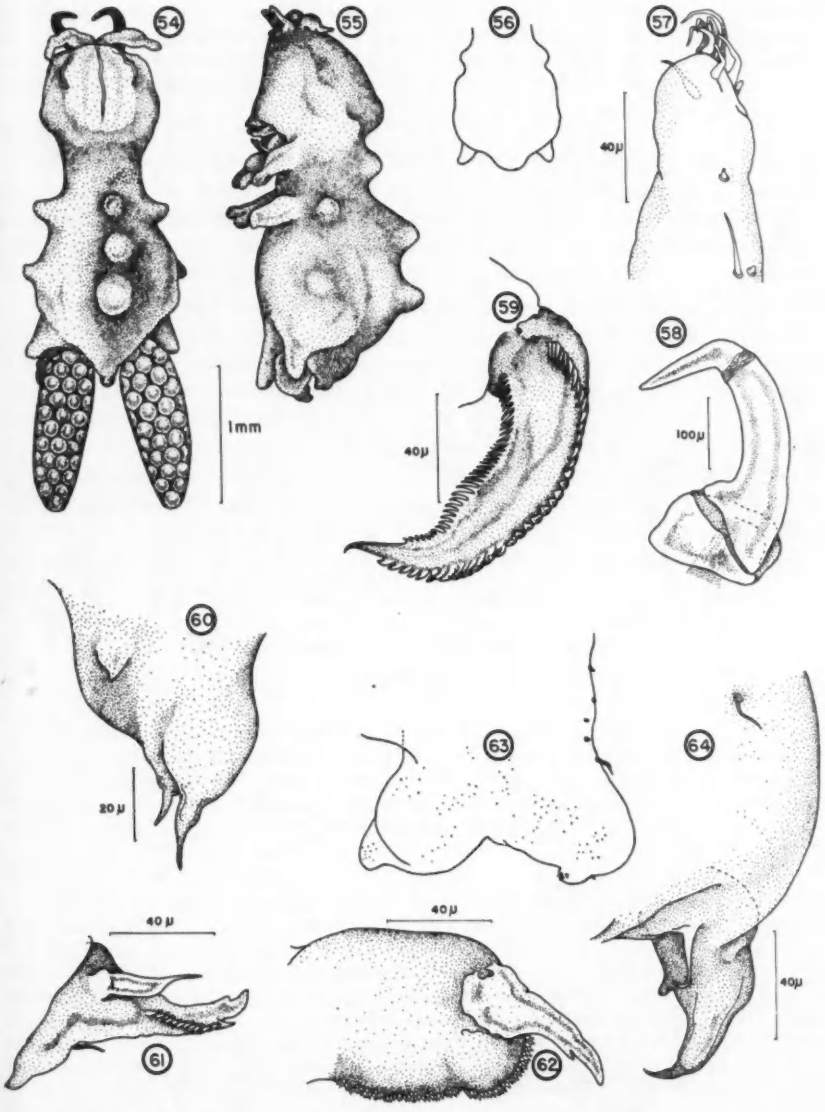
The first antenna bears a fairly abrupt constriction at about two thirds of its length from the base. Its apical armament (Fig. 57) consists of eight setae. Additional armature is present along its anterior margin and it includes the typical double (cone and seta) element seen in Fig. 57. The second antenna (Fig. 58) bears a transverse band of striations at the point of the greatest curvature of its hamulus. The characteristic feature of its mandible (Fig. 59) is the difference between the ventral and dorsal rows of its marginal denticles. The latter are finer and longer than the former. The mandibular palp (Fig. 60), which is very small, has been distorted by handling, as can be seen in the drawing. One can still see, however, that the proximal lobe of its medial margin is pointed and possibly carries a small denticle on its tip. The maxillary claw (Fig. 61) carries a row of 12 denticles on its dorsal margin and is equipped with a single blunt denticle on the ventral one. Its auxiliary spine is fairly short with a sharp, smooth tip. The basal seta is present. The claw of the maxilliped has a single denticle on the concave margin (Fig. 62). The thoracic legs (Fig. 63) are short with only a shallow cleft between the rami. Their surfaces are covered by sparsely and irregularly scattered denticulation. The lateral margin at the base of the exopod carries the usual short seta. In addition, several papillae are present on the proximal part of the lateral margin and four small ones on the tip of the exopod. The globular abdomen is armed with the usual caudal laminae (Fig. 64) which are armed with one dorsal and two ventral setae and on the medial margins with a single conical outgrowth. The dorsal surface of the abdomen, at some distance from the bases of the laminae, bears short, single seta, one of which is illustrated in Fig. 64.

The measurements of the ovigerous specimen (in mm) are as follows: total length — 2.50; cephalothorax length — 0.82; cephalothorax width — 0.82; trunk width — 1.08; egg-sac length — 1.20; egg-sac width — 0.46.

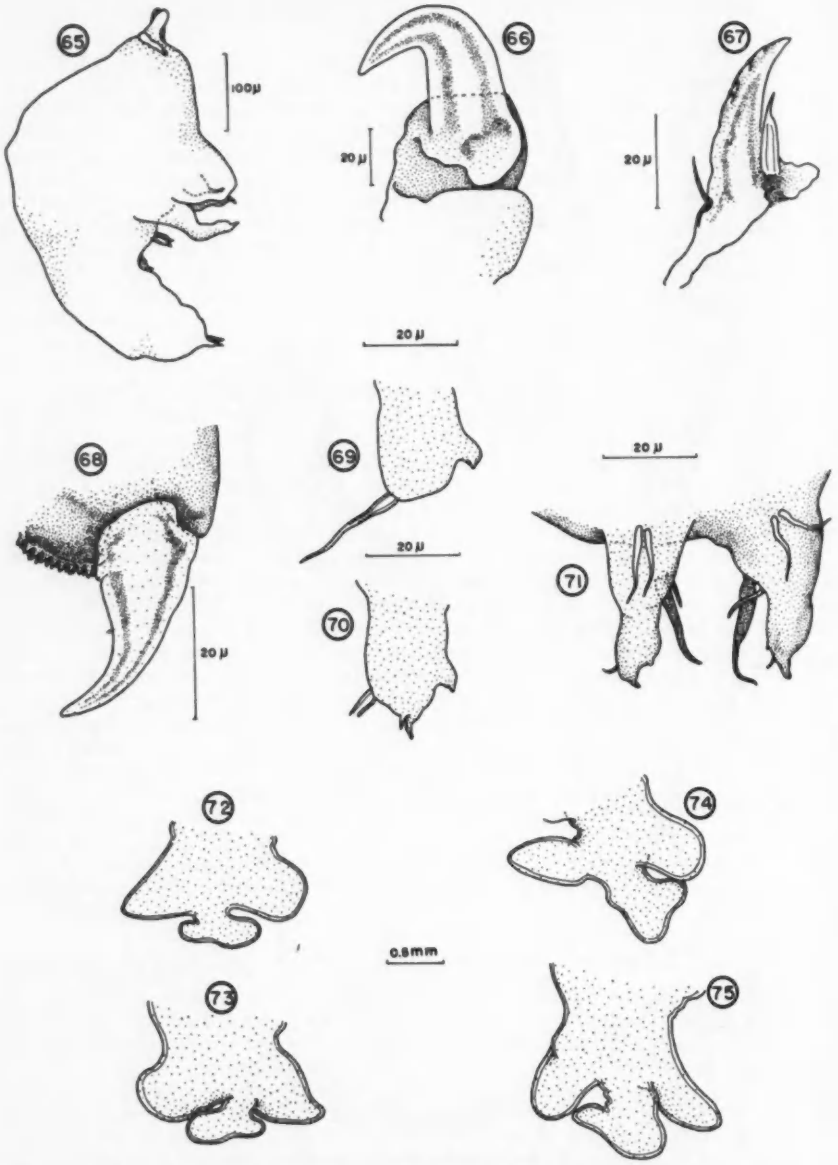
Description of the Male

The general appearance of the male (Fig. 65) is not distinctive enough to be helpful in identification. It is worth noting, however, that although the female belongs to the smallest members of its genus, the size of the males examined is of the usual order of 0.6 mm.

No details were obtained of the structure of the first antenna. The second antenna (Fig. 66) is uniramous, its hamulus apparently without any additional armament. The mandible resembles closely that of the female and the mandibular palp that of the male *Ch. irregularis* (Fig. 10). The maxillary claw (Fig. 67) has only four marginal denticles. Neither the auxiliary spine nor the basal seta offers any distinguishing characters. The claw of the maxilliped (Fig. 68) is devoid of the denticle on the concave margin. The thoracic legs are distinguishable by the small size of the endopod (Fig. 69 and 70). Their lateral margins carry the usual flagellate setae. The rounded tip of the exopod carries two or three (not accurately determined) spinules and a single, papilla-like swelling can be seen at the tip of the endopod. One of the four legs in one male



FIGURES 54–64. ***Chondracanthus pusillus,*** female.
Fig. 54: Entire, dorsal; Fig. 55: Entire, lateral; Fig. 56: Outline of trunk of younger specimen; Fig. 57: First antenna, distal part; Fig. 58: Second antenna, hook; Fig. 59: Mandible, terminal part; Fig. 60: Mandibular palp, ventrolateral; Fig. 61: Claw of maxilla, dorsal; Fig. 62: Maxilliped distal part; Fig. 63: Diagram of thoracopod; Fig. 64: Part of abdomen and caudal lamina.



FIGURES 65–75. ***Chondracanthus pusillus,*** male, ***Ch. zei,*** female, and ***Ch. nodosus,*** female. Fig. 65: *Ch. pusillus*, male entire, outline, lateral; Fig. 66: Second antenna, hook; Fig. 67: Claw of maxilla, dorsal; Fig. 68: Claw of maxilliped; Fig. 69: Abnormal first thoracopod; Fig. 70: Normal first thoracopod; Fig. 71: Caudal furca; Fig. 72 and 73: *Ch. nodosus*, female, left and right first thoracopods; Fig. 74 and 75: *Ch. zei*, female, left and right first thoracopod.

was abnormal (Fig. 69). The tip of its exopod had not developed or was damaged during the parasite's life. The margin of the leg sloped from the lateral seta directly to the endopod and no exopod armament was present. The caudal furca (Fig. 71) is very characteristic. In addition to the normally encountered three setae, its laminae appear to be equipped with two more: one near the base of the dorsal seta and another small setule near the tip. Also, near the tip, the author noticed a small tubercle, the details of which were impossible to clarify.

Discussion

Chondracanthus pusillus appears to have much in common with its larger local relative, *Ch. irregularis*. In many respects its general features seem to parallel those of the larger species. The differences between them are, however, sufficiently great to allow *Ch. pusillus* the status of an independent species. The trunk of the female *Ch. pusillus* has no outgrowth *f* and its outgrowth corresponding in position with *e* of *Ch. irregularis* is different in shape and relative size. There are also differences in the finer details of the appendages (e.g. number of denticles on the claw of the maxilliped). Structural differences between these two parasites are even more evident in the male (maxilliped, thoracic legs, caudal laminae).

It must also be taken into account that *Ch. pusillus* can reach the stage of egg-producing maturity at the size of 2.5 mm. No small specimens of *Ch. irregularis* were ever found bearing egg-sacs and their total length is known to reach 11 mm (Kabata and Gusev, 1966). Although further examination of the new species is desirable, its validity can already be maintained with a reasonable degree of certainty.

Acanthochondria holocephalarum sp.nov.

Syn.: *A. clavata* (Basset-Smith, 1896) of Wilson, 1935

Record of specimens: Nine ovigerous females with males attached were collected by Dr Gordon Bell in Porlier Pass, Strait of Georgia, B.C. on August 1, 1966. One female becomes the holotype of the species and the male attached to it the allotype. Three females with males attached become paratypes. All type specimens are deposited in the National Museum, Ottawa. Host: *Hydrolagus colliei* (Lay and Bennett, 1839). Habitat: Claspers.

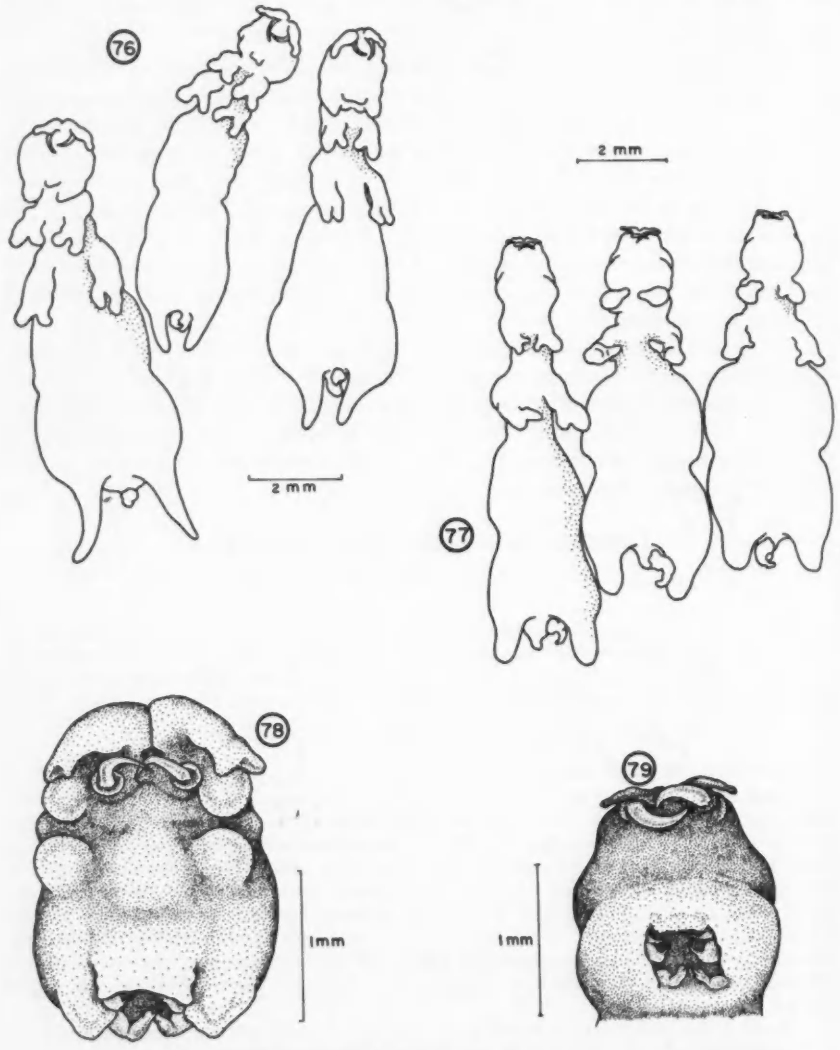
Description of the Female

General appearance (Fig. 77). A typical member of its genus, *A. holocephalarum* exhibits some variations in the shape of its trunk. Figure 77 shows three specimens with an example of these variations. The cephalothorax (Fig. 79) is narrower anteriorly, its anterior margin characterized by the short and slender first antennae. Seen in the ventral aspect the anterior part of the cephalothorax is flat whereas the posterior produces a snout-like, conical swelling with the buccal orifice at its tip. The border between the cephalothorax and the trunk is only slightly notched and the anterior part of the trunk is fairly long with the two pairs of thoracic legs separated by a relatively long distance. The wide trunk proper is separated into two parts by a transverse constriction. The posterior processes of the trunk are short and robust, and end in rounded, blunt tips.

Based on four specimens the measurements (in mm) of the female are: total length — 7.65–8.92; cephalothorax length — 1.49–1.70; cephalothorax width — 1.36–1.61; trunk width — 2.21–2.76.

The first antenna (Fig. 80) narrows rapidly at the tip which is armed with several setae. The author was unable to determine their accurate number. The second antenna is of the usual

type and provides no distinguishing characters. The mandible (Fig. 81) is well developed. Its falcate part is rimmed with marginal denticles which, in this species, appear to be about the same size on both margins. The groove-like surface between the margins is pitted with minute crypts shown as dots in Fig. 81. The mandibular palp (Fig. 82) is distinguished by the absence of a distinct concavity in its medial margin. Its terminal spine is robust and its wall carries two groups



FIGURES 76–79. ***Acanthochondria clavata*** and ***A. holocephalarum***, females.
Fig. 76: *A. clavata*, outline of three specimens in ventral view; Fig. 77: *A. holocephalarum*, outline of three specimens in ventral view; Fig. 78: *A. clavata*, cephalothorax ventral; Fig. 79: *A. holocephalarum*, cephalothorax ventral.

of minute denticles near the margin of the palp on both sides of the terminal spines. The maxillary spine (Fig. 83) is provided with a varying number of denticles on its dorsal margin. In one specimen the left claw had 8 whereas the right claw had 12 denticles. The latter number is, however, more common and occurred in most of the maxillae examined. The auxiliary spine seems to be shorter than usual; the same is true of the basal seta. The claw of the maxilliped (Fig. 84) appears to be devoid of the denticle on the concave margin. Both pairs of thoracic legs are biramous and present no characteristic features. They seem to be devoid of denticulation. The author was unable to determine the details of the caudal furca.

Description of the Male

General appearance (Fig. 85). Neither the habitus nor the size of the male can be used for the purposes of identification. The male resembles other males of the genus and its length is in the order of 0.6 mm.

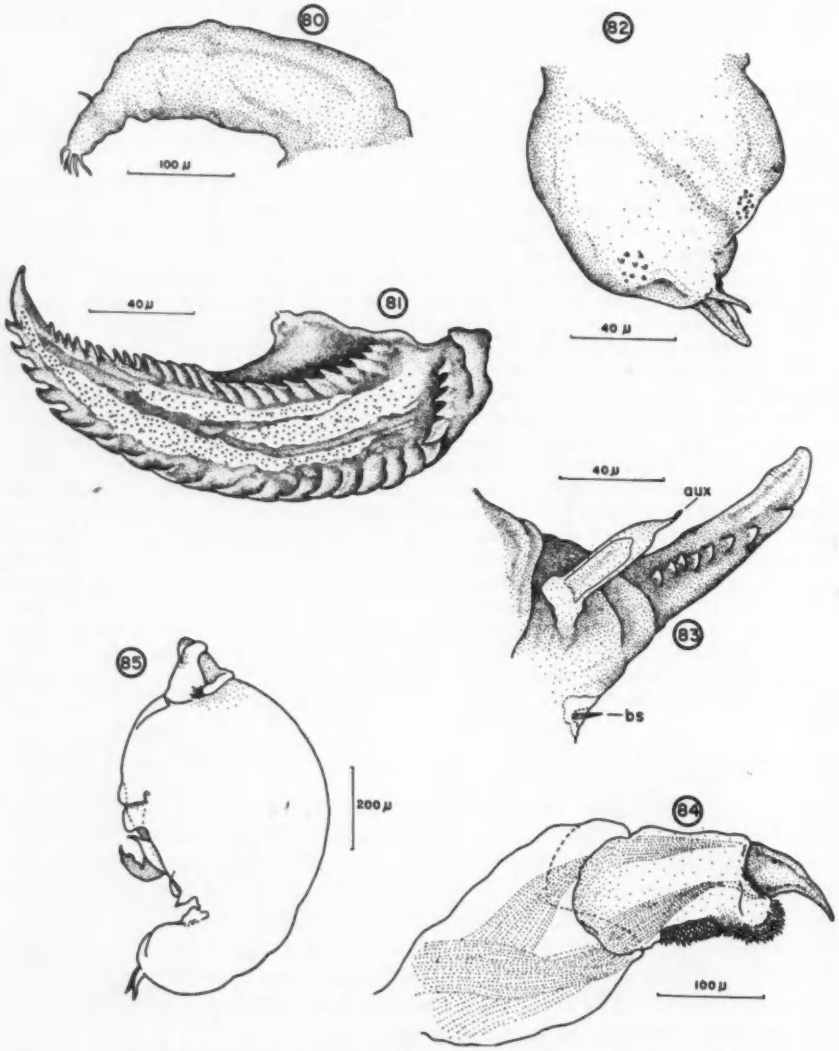
The first antenna (Fig. 86 and 87) is fairly short and slender, wider at the base and tapering towards the apex. The apical armature is frequently damaged as in Fig. 86. When in normal condition (Fig. 87) it consists of eight setae. In this figure the setae which have been circled do not belong to the apical armament. The second antenna is hamate, uniramous, without distinguishing characters. The mandible (Fig. 88) is characterized by the considerable differences in the size of the marginal denticles. Whereas the ventral margin carries large teeth with gaps between them of unequal length, the dorsal row consists of smaller, closely packed denticles. As in the maxilla of the female the mandible of this male differs in the number of ventral denticles present. It is possible that some specimens had mandibles which suffered loss of teeth in the course of the parasite's life. This is suggested by the fact that the mandibles (such as the one illustrated in Fig. 88) which had fewer denticles had also gaps between them, whereas those with a larger number of teeth had no such gaps. The number of teeth in the ventral row varies from 9 to 16. No details of the mandibular palp were obtained. The maxillary claw (Fig. 89) is short, noticeably curved and devoid of denticles. In one specimen a very small protuberance was observed on the convex margin of the claw but others appeared to be quite smooth. Both the auxiliary spine and the basal seta are present. The claw of the maxilliped (Fig. 90), in addition to the small denticle on the concave margin, is distinguished by the presence of a transverse row of small prickles (about 10) near its base. Both pairs of thoracic legs are of the same structure (Fig. 91 and 92). The endopod is rounded and prominent, a small subterminal swelling sometimes being present near its margin. The exopod is more slender and narrower at the tip which is armed with three spines: one of these is long and tapering whereas the other is shorter, more robust, and appears to have a common base with a third, short, and slender spine. The lateral margin has the usual flagellate seta. The author was unable to study the structure of the caudal furca.

Discussion

Wilson (1935) reported *Acanthochondria clavata* (Bassett-Smith, 1896) from the claspers of *Hydrolagus colliei* caught in Pacific Grove, California. His record contained no description or illustrations and asserted close resemblance between the specimens from *Hydrolagus* and *A. clavata*. Wilson's identification, in view of our knowledge of *A. clavata*, cannot be accepted. Since the time of its description, *A. clavata* has never been found outside the North Sea area nor on a host other than the type host *Microstomus kitt* (Walbaum). Its occurrence in the Pacific and on a host taxonomically remote from the type host would be at least surprising.

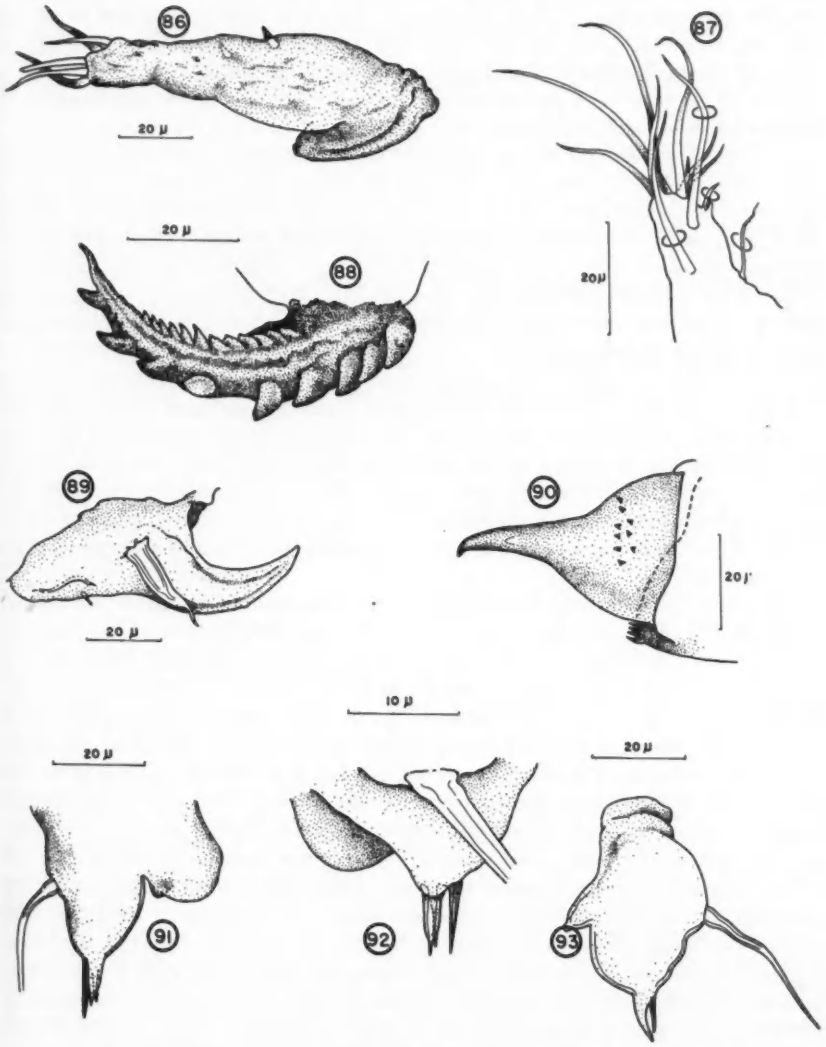
The author made a close comparison between the specimens from *Hydrolagus* and *A. clavata* from the type host and locality. The general appearance of *A. clavata* is illustrated in Fig. 76. A broad resemblance between the two

sets of specimens is clearly shown by Fig. 76 and 77. Even the gross morphology of these specimens is sufficiently different, however, to doubt whether they can be conspecific. One such difference is in the shape and relative size of the posterior processes of the trunk which are more slender and longer in *A. clavata*.



FIGURES 80–85. ***Acanthochondria holocephalarum***, female and male.
Fig. 80: Female, first antenna, entire; Fig. 81: Mandible, distal part; Fig. 82: Mandibular palp, ventral; Fig. 83: Claw of maxilla, dorsal; Fig. 84: Maxilliped entire, dorsal; Fig. 85: Male, entire, outline, lateral. *aux* — auxiliary spine; *bs* — basal seta.

A closer look at the cephalothorax shows quite clearly the differences between them. Whereas the cephalothorax of the parasite of *Hydrolagus* (Fig. 79) has a smooth anterior part of the ventral surface and a conical posterior part,



FIGURES 86–93. ***Acanthochondria holocephalarum*** and ***A. clavata***, males.
Fig. 86: *A. holocephalarum*, first antenna with damaged apical armature; Fig. 87: First antenna, apex with armature; Fig. 88: Mandible, distal part; Fig. 89: Claw of maxilla; Fig. 90: Claw of maxilliped; Fig. 91: First thoracopod, distal half, ventral; Fig. 92: Thoracopod, tip, lateral; Fig. 93: *A. clavata*, thoracopod ventral.

that of *A. clavata* (Fig. 78) carries three pairs of tubercles on the lateral margin of the anterior part. Two of these tubercles are quite distinct and ventral whereas more dorsally situated is a third, smaller, and less distinct pair. Another outstanding difference shown in these figures is the relative size and shape of the first antennae. Those of the parasite from *Hydrolagus* are short and slender; those of *A. clavata* are large and grossly inflated with the distal part marked off so distinctly as to make them appear almost biramous. The buccal orifice of *A. clavata* is not raised on the tip of a tubercular swelling and is displaced in a posterior direction.

The comparison of the fine details of the mouth parts and other appendages of the female revealed no perceptible differences. Some differences were found, however, in the structure of the male appendages. The claw of the maxilliped of *A. clavata* does not have the transverse row of denticles discovered in the parasite of *Hydrolagus*. The thoracic legs of *A. clavata* (Fig. 93) are distinguished by a much smaller size of endopod which is narrow and tapering. On the other hand the exopod is broader with a curving medial margin and with only two terminal spines.

It has been stated in the introduction to this paper that the most reliable distinguishing character of the members of Chondracanthinae is the structure of the trunk (number, size, shape, and arrangement of outgrowths). The genus *Acanthochondria* is devoid of all trunk processes with the exception of the posterior ones. It is, therefore, rather more difficult to distinguish between the species of *Acanthochondria* than between those of the related genera. Inevitably, misidentifications must occur and the present example illustrates them convincingly.

Among the currently known species of *Acanthochondria* there are several which bear a close resemblance to *A. holocephalarum*. They are: *A. constricta* Shiino, 1955; *A. glandiceps* Shiino, 1955; *A. purpurea* Oakley, 1930; and, to some extent, also *A. grandigenitalis* (Yu and Wu, 1932). To obtain incontrovertible proof of significant differences between these species and *A. holocephalarum* (and, indeed, among all those species themselves) it would be necessary to subject them to close scrutiny of the type carried out in the course of this work. Even without such scrutiny one can confidently claim for *A. holocephalarum* the status of an independent species. What is known about the biology and ecology of the genus (Kabata, 1959) suggests that its members are not likely to command wide ranges of hosts or a variety of habitats. Hence a species occurring on a holocephalan host is not likely to have many affinities with other morphologically similar species. When one adds to this the fact that *A. holocephalarum* is the only *Acanthochondria* associated with the external genitalia rather than the branchial region of its host, its unique character is demonstrated fairly conclusively. Under the circumstances the author has no hesitation in describing it as a species previously unrecognized.

ACKNOWLEDGMENTS

The author wishes to place on record his gratitude to Dr H. P. Arai and to Dr Gordon Bell who collected some of the specimens described in this paper.

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Decapod Crustacea from the Queen Elizabeth and Nearby Islands in 1962[1]

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ABSTRACT

Five surveys in the Queen Elizabeth and nearby islands during the summer of 1962 collected 1221 specimens of decapod Crustacea. These decapods comprised 10 species of hippolytid and crangonid shrimps. Sizes and maturity status of specimens indicated variability in adaptation of different species to the low temperature environment. Records of northern distribution of the species in Canada and Greenland are reviewed. Food was mostly detritus and phytobenthos but some species showed preference for foraminiferans or crustaceans.

INTRODUCTION

Ten species of decapod Crustacea were taken in the Queen Elizabeth and nearby islands in 1962 by five surveys of the Arctic Unit of the Fisheries Research Board of Canada in the summer of 1962 (Anon., 1963, Fig. 1). These were *Eualus fabricii*, *E. gaimardi*, *Spirontocaris spinus*, *S. phippsi*, *Lebbeus groenlandicus*, *L. polaris*, *Sabinea septemcarinata*, *Argis dentata*, *Sclerocrangon boreas*, and *S. ferox*. This paper gives these new records and also gives information on the biology of some species to indicate variation in the adaptability of different species to a low temperature environment.

Heegaard (1941) reviewed records of decapod crustaceans from the "Polar Archipelago of North America" and listed eight species as follows: *Lebbeus groenlandicus*, *L. polaris*, *Spirontocaris spinus*, *S. phippsi*, *Eualus gaimardi*, *Sabinea septemcarinata*, *Sclerocrangon boreas* and *Hyas coarctatus*. These records were of specimens collected by Ross and Sabine throughout the archipelago (von Hofsten, 1916) and by Capt. Robert A. Bartlett from Coburg Island and the neighbouring Jones Sound south of Ellesmere Island (Van Winkle and Schmitt, 1936). Heegaard shows map records in the Archipelago of all these species except *H. coarctatus* but this species was listed because it was recorded as far north as 71°21′N in west Greenland (Stephensen, 1935). *Sclerocrangon ferox* and *L. polaris* had been taken also at stations of the Godthaab Expedition in Jones Sound near Coburg Island (Stephensen, 1935). The approximate localities recorded on Heegaard's map or documented by Van Winkle and Schmitt and Stephensen are mentioned in this paper under the species in question.

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[1]Received for publication August 2, 1967.

J. Fish. Res. Bd. Canada, 25(2): 347–362, 1968.
Printed in Canada.

FIG. 1. Place names used in the text.

The surveys of 1962 in the Queen Elizabeth Islands (Fig. 2; Table I) may be briefly outlined as follows:

(*a*) Fifty-one hauls at 17 stations off Allen Bay near Resolute, south of Cornwallis Island (area E: decapods at 10 stations); and five hauls at five stations in Penny Strait north of the island (area D: decapods at two stations). These hauls were made during June 28–August 14.

(*b*) Fifty-nine hauls at four stations in Slidre Fjord near Eureka, Ellesmere Island (area B: decapods at three stations), three hauls at one station in Nansen Sound (area A: no decapods) and three hauls at a station in Strand Fjord, Axel Heiburg Island (area C: no decapods). These hauls were made between July 2 and August 16.

(*c*) Eleven hauls at eight stations in Prince of Wales Strait (area H: decapods at four stations) and one haul at a station in M'Clure Strait (area F). These were made during July 24–August 5.

(*d*) Seventy hauls at 20 stations in Creswell Bay, Somerset Island (area J: decapods at 15 stations). These were made during June 23–August 13.

(*e*) One haul in Cambridge Bay (area L) and five hauls at four stations in nearby Wellington Bay, Victoria Island (area K). These were made during August 27–September 10.

METHODS AND HYDROGRAPHY RELATED TO THE COLLECTIONS

Five shore parties did the collecting, hydrography, etc., by using small outboard-powered boats. The gear used included small otter trawls, dredges,

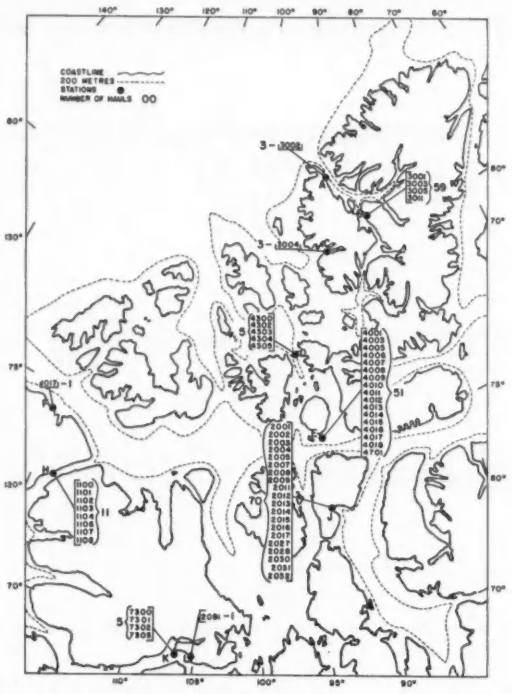

FIG. 2. Station numbers, area letters, and numbers of hauls where collections of decapod Crustacea were or could have been made with the gear used in 1962.

grabs, and bottom sleds, and on two occasions a single specimen (of *S. septemcarinata*) was obtained from a gill net (Table I). Methods in routine biological examination of the specimens were similar to those used previously (Squires, MS, 1965).

About 25% of the hauls were made at depths less than 20 m. Of the others only a few were as deep as 70 m. Temperatures of bottom water where most species were taken at the time of capture were between −1.7 and 2.6 C (Tables I and III). Only a few bottom water temperatures above 1 C were recorded.

SYNOPSIS AND DISCUSSION OF THE SPECIES COLLECTED

Family Hippolytidae

Eualus fabricii (KRØYER)

Only three specimens were taken (Table I). They were from the two most southerly areas, K and L, only (Fig. 2 and 3). This species had previously been recorded at about 70°N in Foxe Basin (Squires, 1966) but not from the Queen Elizabeth Islands.

TABLE I. List of stations with numbers of specimens of each decapod crustacean species collected in the Queen Elizabeth and nearby islands in 1962.

Station No.	Gear[a]	Species[b]										Depth (*m*)	Temp (*C*)	Date	Hauls
		Ef	*Eg*	*Ssp*	*Sp*	*Lg*	*Lp*	*Ss*	*Ad*	*Sb*	*Sf*				
1017	OT	–	–	–	–	–	–	1	–	–	–	50+	–1.0	Aug. 5	1
1100	BS	–	–	–	–	–	–	1	–	–	–	20	–	July 27	1
1101[c]	OT	–	–	–	–	–	–	–	–	–	–	4	–	" 24	1
1102[c]	OT	–	–	–	–	–	–	–	–	–	–	2	–	" 24	1
1103	OT	–	1	–	–	–	–	4	–	–	–	26	–	" 24	1
1104[c]	BS	–	–	–	–	–	–	–	–	–	–	0.3	–	" 25	1
1106[c]	OT	–	–	–	–	–	–	–	–	–	–	7	–	" 26	3
1107	OT	–	12	–	2	–	2	26	–	–	–	16	1.4	" 26	2
1108	OT	–	–	1	–	–	–	–	–	–	–	50	–	" 26	1
2001[c]	Gr	–	–	–	–	–	–	–	–	–	–	10	–	June 25, 26	7
2002	Gr, Dr	–	1	–	–	–	–	18	–	–	–	33	–1.5	June 23–30 July 7–21, Aug. 4, 11	16
2003	Gr, Dr	–	–	–	–	–	–	15	–	1	–	34	–1.6	June 27–29, July 23	7
2004	Gr	–	–	–	1	–	–	–	–	2	–	9	–1.1	June 29	5
2005[c]	Gr	–	–	–	–	–	–	–	–	–	–	10	–	" 29	5
2007	Dr	–	–	–	–	–	1	–	–	–	–	30–0	–1.7	July 2–3, Aug. 13	5
2008	Gr, Dr	–	–	–	–	–	–	12	–	–	–	20–45	–1.7	July 3–25	7
2009	Dr	–	2	–	–	–	1	16	–	–	–	35	–1.6	" 25	1
2011	Dr	–	–	–	–	–	–	117	–	1	–	40	–1.7	" 18–24	2
2012	Dr	–	–	–	–	–	–	34	–	–	–	36	–1.6	" 25	1
2013	Dr	–	–	–	–	–	–	2	–	–	–	15–20	–1.4 to –1.3	" 25	2
2014	Dr	–	–	–	–	–	–	3	–	–	–	35	–1.6	" 25	1
2015[c]	Dr	–	–	–	–	–	–	–	–	–	–	6–15	–	" 30	1
2016[c]	Dr	–	–	–	–	–	–	–	–	–	–	15	–	" 30	1
2017	Dr	–	–	–	1	9	85	–	–	1	–	11–24	–0.6 to 2.6	July 30 and Aug. 6–9	3
2027	Dr	–	–	–	–	1	–	–	–	–	–	13	1.2 to 2.1	Aug. 6	1
2028[c]	Dr	–	–	–	–	–	–	–	–	–	–	26	–	" 6	1
2030	Dr	–	1	–	–	–	–	4	–	–	–	24–27	–1.0	" 9	1
2031	Dr	–	1	–	–	–	2	1	–	–	–	40	–1.0	" 11	1
2032	Dr	–	4	–	1	–	–	13	–	–	–	59–62	–1.0	" 11	2
2081	OT	2	1	–	–	–	–	–	–	–	–	–	–	Sept. 10	1

3001	Dr, BS, Gr OT	–	–	–	–	–	–	58	–	–	3	2–70	–1.6	July 2–Aug. 16	44
3002[c]	Dr, BS	–	–	–	–	–	–	–	–	–	–	29	–	July 11	3
3003	Dr, BS, Gr	–	–	–	–	–	–	1	–	–	–	2–30	–1.6	July 17, Aug. 5	12
3004[c]	Dr, OT	–	–	–	–	–	–	–	–	–	–	2–57	–	July 18	3
3005	OT	–	–	–	–	–	–	21	–	–	–	20–40	–1.5	July 25	2
3011[c]	Dr	–	–	–	–	–	–	–	–	–	–	30–42	–	Aug. 10	1
4001	OT	–	25	2	–	–	213	–	–	–	–	35–52	0.0	Aug. 6	2
4003	OT, Dr Gr, BS	–	11	–	10	1	66	1	–	8	–	10–30	–1.1 to 1.2	June 28–Aug. 14	31
4005[c]	OT	–	–	–	–	–	–	–	–	–	–	0–2	–	July 7–Aug. 3	1
4006	OT, Gr, BS	–	15	–	3	–	57	12	–	30	–	5–15	1.9, 0.6	July 9–Aug. 4	4
4007	OT	–	2	–	–	–	5	–	–	3	–	10	–0.1	July 17	1
4008[c]	OT	–	–	–	–	–	–	–	–	–	–	13	–	" 19	1
4009	OT	–	–	–	–	–	22	–	1	10	–	10	0.4	" 25	1
4010[c]	OT	–	–	–	–	–	–	–	–	–	–	6	–	" 25	1
4011[c]	OT	–	–	–	–	–	–	–	–	–	–	18	–	" 25	1
4012	OT	–	–	–	–	–	5	–	–	2	–	24	0.1	" 25	1
4013	OT	–	–	–	–	–	13	–	–	19	–	6	0.9 to 1.4	" 25	1
4014[c]	OT	–	–	–	–	–	–	–	–	–	–	–	–	" 25	1
4015	OT	–	1	–	–	1	16	–	–	12	–	–	–	" 25	1
4016	OT	–	19	1	2	13	99	4	–	11	–	–	–	" 25	1
4017	OT	–	–	–	–	–	–	–	–	–	–	8	–	" 25	1
4018	OT	–	–	–	–	–	2	–	–	–	–	4	1.4 to 1.5	" 28	1
4300[c]	OT	–	–	–	–	–	–	–	–	–	–	5	–	" 14	1
4302[c]	OT		–	–	–	–	–	–	–	–	–	5	–	" 15	1
4303[c]	OT	–	–	–	–	–	–	–	–	–	–	5	–	" 15	1
4304	OT	–	–	–	1	–	1	–	–	–	–	25	0.0 to 0.2	" 16	1
4305	OT	–	1	–	–	–	1	–	–	–	–	40	–0.2	" 16	1
4701[c]	BS	–	–	–	–	–	–	–	–	–	–	1	–	" 25	1
7300	OT	1	–	–	–	–	2	8	1	–	–	30	1.4	" 23–27	2
7301	OT	–	–	–	–	–	–	1	–	–	–	–	0.1 to 1.4	" 23	1
7302	OT	–	–	–	–	–	–	1	–	–	–	50–60	–	" 23	1
7305[c]	OT	–	–	–	–	–	–	–	–	–	–	20–30	–	" 24	1
Totals	1222	3	97	4	21	25	593	374	2	100	3				209

[a]OT= otter trawl, BS= bottom sled, Gr= grab, Dr= dredge.

[b]*Ef= Eualus fabricii*; *Eg= E. gaimardi*; *Ssp= Spirontocaris spinus*; *Sp= S. phippsi*; *Lg= Lebbeus groenlandicus*; *Lp= L. polaris*; *Ss= Sabinea septemcarinata*; *Ad= Argis dentata*; *Sb= Sclerocrangon boreas*; *Sf= S. ferox.*

[c]No decapod crustaceans were taken.

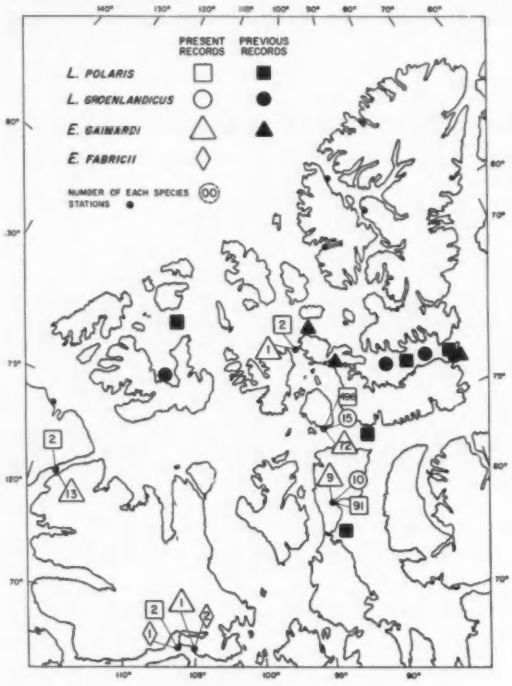

FIG. 3. Distribution records of *Lebbeus polaris*, *L. groenlandicus*, *Eualus gaimardi*, and *E. fabricii* in the Queen Elizabeth Islands.

The specimens were small adult males with immature gonads (Table II). Since this is generally a shallow water species, its northern distribution may be assumed to reach a limit in this area and in Foxe Basin. Depth recorded was 30 m and temperature 1.4 C (Tables I, III).

Eualus gaimardi (H. MILNE-EDWARDS)

There were 97 specimens (37 M, 57 F, 3 not sexed) collected from areas D, E, H, J, and L, most of the specimens being from 6 out of 17 stations at area E (Table I; Fig. 2 and 3). Depths recorded were 5–62 m and temperatures −1.6 to 1.4 C. Previously this species had been recorded from Jones Sound and near Coburg Island (Van Winkle and Schmitt, 1936), and from Belcher and Wellington Channels (Heegaard, 1941).

Although some specimens had a pronounced lobe with a hook on the 3rd abdominal segment, there were more without such a lobe and some had a half-formed lobe intermediate between the two types. All were assigned to the above species.

TABLE II. Maturity status of male and female decapod crustaceans collected in the Queen Elizabeth and nearby islands in 1962. CL = carapace length; *Vd* = vas deferens.

Species	Males				Females				
					Nonovigerous				
	Juveniles	*Vd* small	*Vd* large	CL at 1st maturity (*mm*)	Small ova	Large ova	Ovigerous	CL at 1st maturity (*mm*)	Adults potentially ovigerous annually (%)
Eualus fabricii	–	3	–	–	–	–	–	–	–
E. gaimardi	4	30	6	5	48	–	4	11[a]	67
Spirontocaris spinus	–	–	1	8	2	–	1	–	–
S. phippsi	–	1	4	5	6	6	3	6	64
Lebbeus groenlandicus	–	3	8	11	7	5	2	14	64
L. polaris	11	71	148	7	176	95	67	9	47
Sabinea septemcarinata	163	50	15	10	100	12	11	12	72
Argis dentata	–	–	–	–	1	–	–	–	–
Sclerocrangon boreas	5	9	23	13	49	8	3	23	83
S. ferox	–	2	–	–	1	–	–	–	–

[a]Doubtful. Samples were small and may not be representative.

TABLE III. Sizes of male and female decapod crustaceans collected in the Queen Elizabeth and nearby islands in 1962. CL = carapace length.

Species	Males			Females			Depth range (*m*)	Temp range (*C*)
	No. examined	Cl (*mm*) Range	Avg	No. examined	Cl (*mm*) Range	Avg		
Eualus fabricii	3	7–9	8	–	–	–	30	1.4
E. gaimardi	37	5–11	7	57	3–14	8	0–62	−1.6 to 1.9
Spirontocaris spinus	1	8	–	3	7–14	9	35–52	0.0
S. phippsi	5	5–7	6	16	4–10	8	0–62	−1.1 to 2.6
Lebbeus groenlandicus	11	11–15	13	14	5–25	16	10–30	−1.1 to 2.6
L. polaris	226	5–13	10	349	4–16	11	0–52	−1.7 to 2.6
Sabinea septemcarinata	66	5–13	8	126	4–19	9	2–62	−1.7 to 1.9
Argis dentata	–	–	–	1	14	–	10–30	0.4 to 1.4
Sclerocrangon boreas	32	5–18	13	61	7–28	17	6–40	−1.7 to 2.6
S. ferox	2	6,10	8	1	17	–	2–70	−1.6 to −1.1

The percentage (67%) potentially ovigerous annually (Table II; see also Squires, 1967a) indicates a moderate degree of adaptation to Arctic conditions but the small proportion of mature specimens in this collection may indicate slow growth and late maturity as a result of the prevailing low temperatures (Squires, MS, 1965). The data on lengths of these specimens (Table III; Fig. 6) show that they are smaller than those collected from Ungava Bay and Foxe Basin (Squires, 1957; MS, 1965), but length at first maturity (11 mm) was only 1 mm greater than in specimens of this species from Ungava Bay (Squires, 1957). The four ovigerous females had eggs with undeveloped embryos (Table IV) indicating that they had been extruded just previous to the date of capture, July 26.

TABLE IV. Egg diameters and gross embryo development of decapod crustaceans collected in the Queen Elizabeth and nearby islands in 1962.

Species	Eggs eyed			Eggs not eyed		
	Egg diam. range (*mm*)	No. examined	Dates	Egg diam. range (*mm*)	No. examined	Dates
Eualus gaimardi	–	–	–	1.0–1.2	4	July 26
Spirontocaris spinus	–	–	–	1.4	1	Aug. 6
S. phippsi	–	–	–	1.5	3	June 28–Aug. 7
Lebbeus groenlandicus	–	1	July 25	2.5	1	July 25
L. polaris	2.0–2.2	17	June 28–Aug. 6	1.8–2.2	51	July 13–Aug. 23
Sabinea septemcarinata	–	2	July 23–Aug. 23	1.8–2.0	9	July 22–Aug. 23
Sclerocrangon boreas	3.2–3.5	2	July 6–13	3.0	1	July 25

Spirontocaris spinus (SOWERBY)

Only four specimens (1 M, 3 F) of this species were taken, by otter trawl at area E (two stations) and area H (Table I; Fig. 2 and 4). Previously this species had been recorded from Coburg Island and in Jones Sound (Van Winkle and Schmitt, 1936), and near the south end of Melville Island and in Prince of Wales Strait (Heegaard, 1941).

One female, 14 mm in carapace length, was ovigerous (Table II). Embryos were undeveloped (Table IV). The depths trawled were 35–52 m where these specimens were taken (Table III), and no hauls were made deeper than 70 m, so it is possible that this species may be present at greater depths throughout the area. Temperature recorded was 0 C.

Spirontocaris phippsi (KRØYER)

There were 21 specimens (5 M, 16 F) collected from areas D, E, J and H. Most of the specimens were from four stations at area E (Table I; Fig.

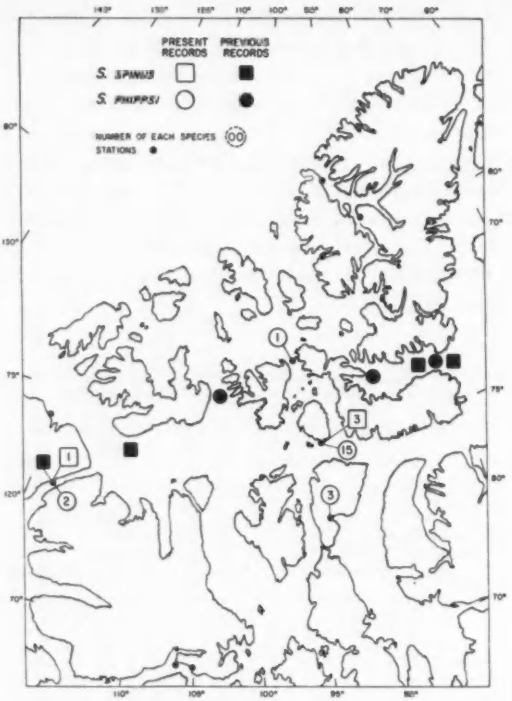

FIG. 4. Distribution records of *Spirontocaris spinus* and *S. phippsi* in the Queen Elizabeth Islands.

2 and 4). Depths recorded were 5–62 m and temperatures –1.1 to 2.6 C. Previously this species had been recorded from Jones Sound and east of Melville Island (Heegaard, 1941).

Of the females, 60% were potentially ovigerous annually indicating moderate adaptation to low temperatures. First maturity occurred at a relatively small size (6 mm CL, Table II). Males and females reached a maximum length (Table III; Fig. 6) similar to those from other northern areas (Squires, 1965). Embryos were undeveloped in the three ovigerous females examined (Table V).

Lebbeus groenlandicus (FABRICIUS)

There were 25 specimens (11 M, 14 F) taken at areas E (at 3 stations) and J (at 2 stations) (Fig. 2 and 3). Most specimens were taken by otter trawl at area E (Table I). Depths recorded were 10–30 m and temperatures –1.1 to 2.6 C. Previously this species had been recorded from Coburg Island (Van Winkle and Schmitt, 1936) and from Jones Sound and Hecla and Griper Bay (Heegaard, 1941).

TABLE V. Food items in decapod crustacean stomachs expressed as a percentage of the total stomachs examined for each species collected in the Queen Elizabeth and nearby islands in 1962.

	Food item[a]												Total stomachs examined
	E	Ph	C	Cop	Ost	Eup	P	F	SS	Ga	K	Pe	
Eualus fabricii	50	50	–	–	–	–	–	–	–	–	–	–	2
E. gaimardi	78	20	2	2	–	–	–	1	–	–	–	–	86
Spirontocaris spinus	25	25	–	–	–	–	–	75	–	–	–	–	4
S. phippsi	57	43	–	–	5	–	–	19	–	–	–	–	21
Lebbeus groenlandicus	33	67	4	–	8	8	–	12	–	4	4	8	25
L. polaris	73	27	3	0.2	1	1	0.3	6	–	0.2	–	–	550
Sabinea septemcarinata	79	21	1	–	–	–	–	–	–	–	–	–	196
Sclerocrangon boreas	76	12	6	–	–	–	7	2	3	1	–	–	95
S. ferox	67	33	–	–	–	–	–	–	–	–	–	–	3
													983

[a]E = empty; Ph = phytobenthos or detritus; C = crustacean; Cop = copepod; Ost = ostracod; Eup = euphausiid; P = polychaete; F = foraminiferan; SS = sponge spicules; Ga = gastrapod; K = seaweed; Pe = pelecypod.

Of the females, 64% were potentially ovigerous annually (Table II), presumably evidence of good adaptation to a cold water area. The maximum sizes of male and female specimens taken (Table III; Fig. 6) were less than for those from Newfoundland and Labrador but apparently as large as for those from other northern areas (Squires, MS, 1965). Embryos were developed to the eye-pigmented stage and were ready to hatch on July 25 in one female examined. Also, one female had eggs with no evidence of embryo development on July 25 (Table IV).

Lebbeus polaris (SABINE)

This was the most abundant species in these collections. There were 593 specimens (230 M, 349 F, 24 unsexed) taken in areas D (at two stations out of five), E (10 stations out of 17), H (one station out of eight), J (4 stations out of 20), and K (one station out of four) (Table I; Fig. 2 and 3). Out of these, 496 specimens were taken at area E. Depths recorded were 0–52 m and temperatures −1.7 to 2.6 C. Previously this species had been recorded from Coburg Island, 2.5–20 fath; and Jones Sound, 7–20 fath (Stephensen, 1935; Van Winkle and Schmitt, 1936), and also from Hazen Strait, Barrow Strait, and southeast of Somerset Island (Heegaard, 1941).

Of the females, 48% were potentially ovigerous annually (Table II). This suggests that egglaying may be biennial and that this species has only fair adaptation to the low temperatures and long winters of this area. Most females had eggs with undeveloped embryos when taken during July 13–August 23 (Table IV).

These specimens (Table III; Fig. 6) were slightly smaller than those from Newfoundland and Labrador but about the same size as those from other northern areas (Squires, MS, 1965).

Family Crangonidae

Sabinea septemcarinata (SABINE)

There were 374 specimens (288 M, 126 F, 20 unsexed) taken in areas B (three out of four stations), E (3 out of 17 stations), H (four out of eight stations), J (11 out of 20 stations) and K (three out of four stations) (Table I; Fig. 2 and 5). Most specimens (235) were taken at area J. Depths recorded were 2–70 m and temperatures −1.7 to 1.9 C. Previously this species had been recorded from Jones Sound and Wellington Channel (Heegaard, 1941).

More than two-thirds of the females of this species were small and apparently juvenile (Fig. 6). (Also about two-thirds of the males taken were juveniles.) Of the mature adult females 72% were potentially ovigerous annually (Table II). Eggs appeared to have been extruded just previous to capture, during July 22–August 23 (Table IV).

Argis dentata RATHBUN

Only two specimens were taken, one at area E and one at area K (Table I; Fig. 2 and 5). These specimens were immature females and may be accidentals

from deepwater populations. Depths recorded were 10–30 m and temperatures 0.4–1.4 C. Previously this species had been recorded from near Coburg Island (Heegaard, 1941).

Sclerocrangon boreas (PHIPPS)

There were 100 specimens taken at areas E and J (Table I; Fig. 2 and 5). However, 95 of these were from 8 stations out of 17 at area E. Almost all were taken at depths less than 30 m and the haul with the greatest number was at 5–15 m. Temperatures recorded were −1.7 to 2.6 C. Previously this species had been recorded from Coburg Island and Jones Sound (Van Winkle and Schmitt, 1936), and M'Clure Strait, Hazen Strait, Belcher Channel, Wellington Channel, Barrow Strait, and Lancaster Sound (Heegaard, 1941).

The percentage of females potentially ovigerous in a year (83%; Table II) suggests that there must be annual spawning by mature individuals. This is evidence of a comparatively high degree of adaptation to a cold-water area.

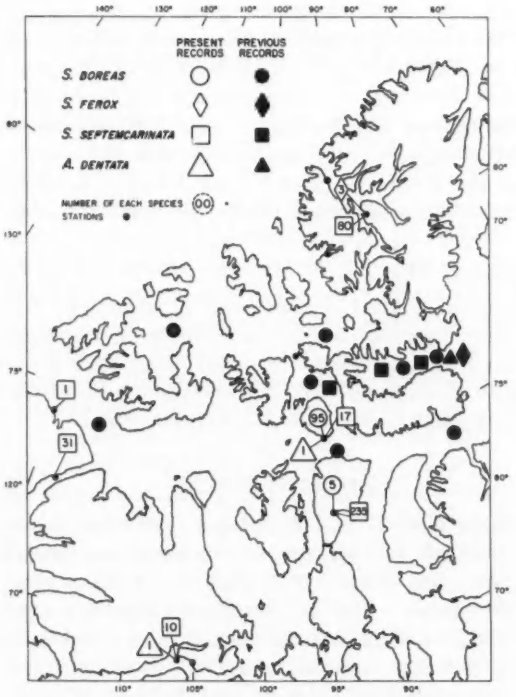

FIG. 5. Distribution records of *Sclerocrangon boreas*, *S. ferox*, *Sabinea septemcarinata*, and *Argis dentata* in the Queen Elizabeth Islands.

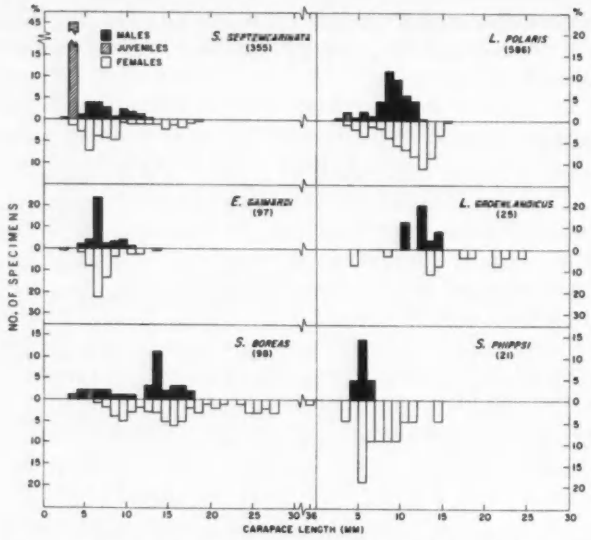

FIG. 6. Length frequencies of six species of decapods from the Queen Elizabeth and nearby islands, 1962.

Embryos in eggs of females taken July 6–13 were ready to hatch but on July 25 one had eggs recently extruded (Table IV).

The sizes of the specimens taken (Table III; Fig. 6) were similar to those from other areas in the northwest Atlantic (Squires, MS, 1965).

Sclerocrangon ferox G. O. SARS

Three specimens (2 M, 1 F) were taken, all at area B (Table I; Fig. 2 and 5). These were juveniles and most likely were accidentals from a deep water population nearby. Depths recorded were 2–70 m and temperature −1.6 C. Previously this species had been recorded from the entrance to Jones Sound by the Godthaab Expedition (Stephensen, 1935).

STOMACH CONTENTS

In most species more than 50% of the stomachs examined were empty (Table V). In all except one species the most frequent food items were phytobenthos and detritus. The one species in question was *S. spinus*, of which, as in other areas where observations have been made (Allen, 1962; Squires, 1962), the stomachs most frequently had foraminiferans. Foraminiferans also occurred but only occasionally in stomachs of *E. gaimardi*, *S. phippsi*, *L. groenlandicus*, *L. polaris*, and *S. boreas*. Crustaceans, including ostracods, euphausiids, and copepods, occurred occasionally in stomachs of *E. gaimardi*, *S. phippsi*, *L. groenlandicus*, *L. polaris*, *S. septemcarinata*, and *S.*

boreas. Small gastropods were present infrequently in stomachs of *L. groenlandicus*, *L. polaris*, and *S. boreas*. Small pelecypods and pieces of seaweed were seen in stomachs of *L. groenlandicus* only, polychaetes in stomachs of *L. polaris* and *S. boreas* and sponge spicules in *S. boreas* only. Almost all stomachs had small amounts of fine sand.

GENERAL DISCUSSION

Maturity status of gonads as an indication of cold adaptation. Squires (MS, 1965; 1967a and b, in press) suggests that in decapod crustaceans the percentage of females potentially ovigerous annually will indicate whether or not individuals in a population are under stress because of prevailing low temperatures. If the percentage is low in a random sample from a population, it is possible that the decapods in question (which may individually spawn once, twice, or three times in a lifetime) may only spawn in alternate years or once only at a large size and a greater than usual age. Generally, random samples from shrimp populations yield some immatures of possibly 1 year of age and also representative numbers from a few other year-classes. Failures of year-classes to survive in usual numbers must be exceptional since I have not detected any such failures in the many samples of various species of shrimps that I have examined. Therefore, in calculations of percentages ovigerous in samples, bias caused by large numbers of any one year has not been apparent. Such calculations are based on mature animals since the usual sampling gear does not capture juvenile animals of the 1st year. In the present samples, however, juveniles were present but they were ignored in the calculations of percentages potentially ovigerous.

In the 10 species examined from the Queen Elizabeth Islands, only 6 had sufficient (but low) numbers of females available for assessment of maturity status. At the time of capture eggs would be either carried and ready for hatching or just hatched, or recently extruded and showing no signs of embryonic development. Also, in nonovigerous females the ova in the ovaries would be either small and not likely to be extruded before a year or more had elapsed, or large and likely to be extruded in a comparatively short time. In four of the six species, *L. groenlandicus*, *L. polaris*, *S. septemcarinata*, and *S. boreas*, some specimens had eggs with late embryos (eye pigment present) (Table IV) suggesting that hatching would take place at least up to August 6 (the latest date these were taken). But in these and in *E. gaimardi*, *S. spinus*, and *S. phippsi* individual specimens had eggs with early embryos (no eye pigment present) indicating that egg extrusion had occurred from July 13 to August 23, the dates of the samples from which they were obtained. The species *E. gaimardi*, *S. phippsi*, *L. groenlandicus*, *S. septemcarinata*, and *S. boreas* appeared to have good adaptation to the low temperatures and long winters of the Arctic since most mature individuals appeared to spawn annually for the two or three times that they probably spawn in a lifetime.

Carapace lengths at first maturity.— Decapod Crustacea from low temperature areas generally show first signs of maturity at a larger size than those

from moderate temperature areas. For example, in Frobisher Bay, specimens of several species were first mature at lengths appreciably greater than in Ungava Bay or in Newfoundland (Squires, 1961). This generalization would probably not apply to species with growth increments greatly reduced by low temperatures over long winter periods. Maturity in such species would probably not occur until they were quite old but still small, and some individuals in very low temperature areas might not mature to reproduce before they died. In the Queen Elizabeth Islands *S. boreas* and *L. polaris* had matured when smaller than or similar in size to individuals of these species from more southerly areas such as Ungava Bay and Newfoundland (Squires, MS, 1965).

Farthest north records.— Since the stations near Eureka (areas A, B, and C; Fig. 1 and 2) are north of the rest of the stations where decapods were recorded from Canada previously, the records of species from A, B, and C are necessarily the farthest north in Canada. These are for *S. ferox* and *S. septemcarinata*. However, Stephensen (1935) and Heegaard (1941) review records, mainly for west Greenland, which are still farther north (most to 82°N latitude) except for *S. ferox* (73°N).

ACKNOWLEDGMENTS

I am indebted to Dr E. H. Grainger of the Arctic Biological Station, Fisheries Research Board of Canada, Ste. Anne de Bellevue, Quebec, for sending me specimens of decapod Crustacea from collections in the Arctic. I am grateful also to Mr G. E. Tucker of the St. John's Station, FRBC, St. John's, Nfld., who did most of the primary biological examinations of specimens for this paper.

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Sub-Zero Preservation of Atlantic Salmon Sperm[1]

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ABSTRACT

Non-frozen storage of sperm for several weeks was achieved by the use of buffered protective agents and low temperature. For example, sperm stored with 5% dimethyl sulphoxide at −4.5 C for 28 days gave 81% fertility; use of 5% ethylene glycol allowed storage at −3 C for 38 days, giving 70% fertility. It is suggested how these results may be employed for interspecific mating of salmonids. Attempts to employ glycerol as a protective agent were unsuccessful as were all attempts at freezing sperm.

The necessity of providing an adequate air-space in vials used for short-term storage of undiluted sperm of Atlantic salmon is demonstrated.

Although a rough correlation between motility of sperm and fertilization is reported, caution in the interpretation of motility as an indication of fertility is suggested.

INTRODUCTION

Successful storage of fish gonadal products is a prerequisite not only for more efficient hatchery operation, but also for effective pisciculture. Short-term storage of fish sperm, of the order of a few days, has been known for over a hundred years (see Barrett, 1951, for review of early literature). More recently, criteria for short-term storage have been discussed (Foerster, 1965; Withler and Humphreys, 1967).

Long-term preservation of living cells usually involves freezing or sub-zero storage in the presence of a protective agent. The use of glycerol (Polge et al., 1949), ethylene glycol (Polge et al., 1949), and dimethyl sulphoxide (Lovelock and Bishop, 1959) for this purpose has allowed successful storage of a variety of biological materials. The experiments reported here were started in 1962 and were designed to obtain long-term storage of Atlantic salmon sperm.

MATERIALS AND METHODS

Atlantic salmon (*Salmo salar*) from River Philip, N.S. hatchery were used. Milt was taken three times from 12 tagged fish at 1-week intervals. The fish were wrapped in damp towels to avoid contamination by water and slime. Eggs were obtained from females at the hatchery. To assess fertility at zero storage time, batches of eggs were fertilized at the hatchery by a sperm sample from each male. Fertilized eggs were packed with water in plastic containers with small holes in the lid; unfertilized eggs were packed in their own egg

[1]Received for publication October 30, 1967.

J. Fish. Res. Bd. Canada, 25(2): 363–372, 1968.
Printed in Canada.

fluid. Sperm and egg samples were transported to the laboratory in an insulated box containing ice. Fresh sperm and eggs were also obtained from fish held in freshwater at the laboratory. In early experiments sperm was stored in capped vials in the dark at 2 C; later samples were stored in small quantities in vials with perforated lids. Diluted sperm (1 ml) stored at subzero temperatures was contained in sealed 20-ml glass vials under oxygen. The undiluted sperm samples were tested daily for 5 days, others at approximately weekly intervals.

Diluents

Alsever's solution (Hodgins and Ridgway, 1964b) contained: glucose, 2.05 g; sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), 0.8 g; sodium chloride, 0.4 g; and water, 100 ml. This solution had a *p*H of 8.2; for some experiments this was adjusted to 7.5.

	Cortland medium (Wolf, 1963) (g)	Modified Cortland medium (g)
Sodium chloride	7.25	1.88
Calcium chloride ($CaCl_2 \cdot 2H_2O$)	0.23	0.23
Potassium chloride	0.38	7.20
Sodium phosphate ($NaH_2PO_4 \cdot H_2O$)	0.41	0.41
Sodium bicarbonate	1.00	1.00
Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	0.23	0.23
Glucose	1.00	1.00

All substances except sodium bicarbonate were dissolved in about 850 ml water. The sodium bicarbonate was dissolved and added separately. The volume was made up to 1000 ml.

Egg-citrate (Salisbury et al., 1948) consisted of sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), 3.5 g, dissolved in 100 ml water and adjusted to *p*H 6.8 with 5% citric acid. An equal volume of hen's egg yolk was added to this.

Pautard's medium (Pautard, 1962) consisted of potassium chloride, 0.3 M; dipotassium monohydrogen phosphate, 0.01 M; and potassium dihydrogen phosphate, 0.005 M. This solution was adjusted to *p*H 6.8 with hydrochloric acid. For some experiments this medium was supplemented with 0.1% glucose.

Protective Agents

Commercial dimethyl sulphoxide (DMS) and glycerol were used without purification; ethylene glycol (EG) was distilled to remove a slight discoloration.

Preparation of Sperm for Cold-Storage

To the sperm was added an equal volume of diluent containing enough protective agent to give the required final concentration. The addition was made gradually over a period of 1 hr. In the case of sperm diluted 10-fold, the sperm was first diluted with 4 volumes modified Cortland medium, followed by a further 5 volumes diluent containing protective agent. A further ½- or

2-hr period of equilibration was allowed. These operations were carried out in the cold room at 2 C. Samples (1 ml) were kept in 20-ml capped vials and, for experiments below 0 C, flushed with oxygen. The vials were placed in polyfoam-lined containers in order to minimize sudden temperature changes.

Freezing of Sperm Samples

Sperm was diluted in the same manner as for non-frozen cold-storage except that a 6-hr equilibration period was allowed. About 0.5 ml diluted sperm was placed in a 1-ml ampoule which was then sealed. Freezing was accomplished as soon as possible after sealing by either direct immersion in liquid nitrogen or by cooling at 1 C per min in a Canalco controlled freezing unit to −30 C and then rapidly to −196 C. Frozen samples were stored in liquid nitrogen for 24 hr.

Fertility Tests

Sperm (1 ml) was added "dry" to batches of between 150 and 250 eggs in beakers in the cold room at 2 C. After 2 min, 150 ml dechlorinated water was added and decanted 2–3 min later. This wash was repeated twice, again at 2 C. The eggs were transferred to Petri dishes and kept in the laboratory hatchery under running dechlorinated water. The temperature of the water dropped from 12 to 6 C between November and January, during which period the experiments were carried out.

Detection of Fertilization

The eggs remained in the hatchery for 7–12 days depending on the water temperature (7 days at 12 C, longer as the temperature dropped). After the incubation period, the dishes were removed from the hatching tank, water decanted from the eggs, and 8% acetic acid added. A characteristic white streak of developing embryo was seen in fertilized eggs but was absent from the others.

Motility of Spermatozoa

Sperm samples were examined microscopically in phase contrast at a magnification of 630×. Motility was induced by dilution of the sperm on a microscope slide with an excess of ice cold 20% seawater. This solution was found to maintain the activity of spermatozoa for over 1.5 hr in a test tube (Ellis and Jones, 1939).

RESULTS

The ability of fresh sperm to fertilize eggs was found to be very high in all batches tested. The proportion of eggs fertilized usually ranged from 95 to 100%. Figure 1 illustrates the decline of fertilizing capacity of sperm on storage at 2 C in filled, sealed vials, as opposed to open or loosely capped vials. Each point represents the average of several determinations, the number

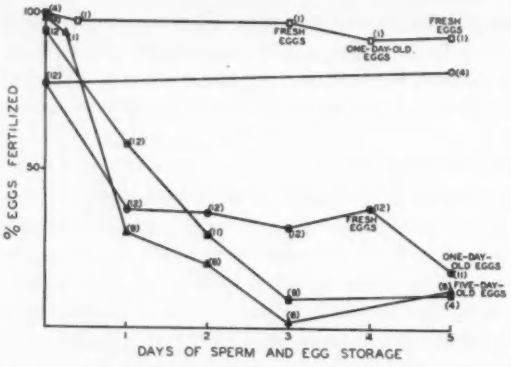

FIG. 1. Fertilization capacity of Atlantic salmon sperm after storage at 2–3 C. Sperm collected on: ●, November 7th, 1963; ■, November 14th, 1963; ○, ▲, November 21st, 1963; □, November 20th, 1964; sperm held in sealed vials (solid points) or in vials with perforated lids (open points); each point represents an average of a number of tests, indicated in parentheses at the relevant point

being indicated in parentheses next to each point. The same fish were used for each series, milt being collected at weekly intervals. Fresh eggs were used on days 0 and 4; 1-day-old eggs were used on days 5 (first series) and 1. Otherwise eggs were the same age as the day number on which they were used.

Storage for longer periods involved the use of extenders or diluting media, and the protective agents DMS, EG, and glycerol. The effect of DMS on sperm storage is shown in Table I. Addition of unbuffered DMS inactivates sperm. Supplementing either Alsever's solution or modified Cortland medium with 12.5% DMS was seen in 1964 to allow 24 hr storage at 2 C with retention of high fertilizing capacity. Diluting the sperm concentration 10 times greatly reduced its potency, and fast deep-freezing completely destroyed the ability to fertilize. Pautard's medium was used with little success.

Whereas in 1964 ½-hr equilibration time was used, this was increased to 2 hr in 1965 for unfrozen samples and to 6 hr for samples subsequently frozen. However, the extended period of equilibration did not produce successful frozen storage. Egg citrate, a common diluent for mammalian sperm, was somewhat less successful for unfrozen storage than either Cortland or Alsever's medium. The following year, variations in the concentration of protecting agent and storage temperature produced a substantially increased storage period. Storage with DMS was successful for 28 days using 5% DMS in modified Cortland medium at –3 C and no tests were done beyond that time.

As shown in Table II, EG was also used successfully as a protecting agent. Early experiments with 12.5% EG in both modified Cortland and Alsever's media gave some retention of fertilizing ability of sperm. As with

TABLE I. Preservation of salmon sperm using DMS.

Year	Diluent	% DMS	Time stored	Storage temp (C)	% fertilization	Time of equilib.
1963	None	10	24 hr	+2	0	½ hr
1964	Alsever's	12.5	24 hr	−196	0	½ hr
		12.5	"	+2	93.5	"
	Modified Cortland	12.5	"	+2	92	"
		12.5	"	+2	2	"[a]
	Pautard's	12	"	+2	6	"
	Pautard's + glucose	12	"	+2	7	"
1965	Alsever's	12.5	3 hr	+2	94.5	2 hr
		12.5	8 "	+2	93	"
	Modified Cortland	12.5	3 "	+2	97	"
		12.5	8 "	+2	98	"
	Egg	12.5	3 "	+2	55	"
		12.5	8 "	+2	58	"
1966	Alsever's	5	21 days	−3, −6.5	0	"
		10	11 "	−3, −4.5	0	"
		10	11 "	−6.5	4	"
		10	21 "	−3, −6.5, −4.5	0	"
		12.5	19 "	−4.5	0	"
		15	22 hr	+2	17	"
		15	22 hr	−13	0.5	"
		15	11 days	−13	0	"
	Modified Cortland	5	22 hr	+2	65	"
		5	22 hr	−4.5	42	"
		5	11 days	−4.5	96	"
		5	21 "	−3	87	"
		5	28 "	−4.5	81	"
		7.5	9, 19, 36 "	−4.5	0	"

[a]One-tenth quantity of sperm.

DMS, reducing the concentration of sperm had a deleterious effect. Similar experiments in 1965 with a longer period of equilibration were more successful. Comparatively long storage of live sperm was carried out in 1966 with 5% EG in Cortland medium at −3 C (70% fertility when last tested at 38 days). Alsever's solution under the same conditions gave 37% fertility.

Glycerol was tested as a protective agent at 12.5% in modified Cortland and Alsever's media; no eggs were fertilized by sperm stored with glycerol in any experiment.

All samples described so far with one exception (1964, 12.5% DMS in Alsever's solution at −196 C) were not frozen. All attempts to freeze sperm samples either rapidly or at a slower controlled rate were unsuccessful. Diluents included the modified Cortland medium, Alsever's solution, Pautard's medium,

TABLE II. Preservation of salmon sperm using EG.

Year	Diluent	% EG	Time stored	Storage temp (C)	% fertilization	Time of equilib.
1963	Modified Cortland	7.5	3 days	+2	18	½ hr
1964	Modified Cortland	12.5	24 hr	+2	5	"
		12.5	"	+2	0.6	"[a]
	Alsever's	12.5	"	+2	24	"
1965	Modified Cortland	12.5	3 hr	+2	100	2 hr
		12.5	8 hr	+2	97	"
1966	Modified Cortland	5	21 days	−3	87	"
		5	21 "	−6.5	0	"
		5	38 "	−3	70	"
		10	21 "	−3	0	"
		10	21 "	−6.5	20	"
		10	21 "	−4.5	7	"
		10	38 "	−3	3	"
		10	28 "	−6.5	6	"
		10	38 "	−4.5	4	"
	Alsever's	5	21 "	−3	2	"
		5	38 "	−3	37	"
		10	21 "	−3	2	"
		10	21 "	−6.5	0	"
		10	21 "	−4.5	1	"
		40	24 hr	+4	0	"
		40	1, 11 days	−26	0	"

[a]One-tenth quantity of sperm

egg-citrate buffer, as well as a skim milk extender used routinely for frozen preservation of bull spermatozoa. All three protective agents, DMS, EG, and glycerol, were added to diluted sperm to give a final concentration equal to 7.5, 10.0, 12.5, 15.0, and 17.5%. Microscopic examination of the sperm suspensions after freezing and thawing did not show motility expected of viable sperm. The protective agent was removed by dialysis from samples of each type before testing for fertility. However, such attempts to reactivate spermatozoa failed and fertility tests showed all samples negative after freezing.

Microscopic examination showed the characteristic ebullient, whirling motility in all samples which subsequently produced a high percentage of fertilization, e.g. 5% DMS in modified Cortland medium at 2 C for 22 hr (65% fertility). Samples with no motion invariably gave no fertilization, e.g. frozen samples. In contrast to the highly fertile samples, some samples contained many sperm which showed a movement slow enough for individual cells to be distinguished. Fertility tests on these samples were much lower,

e.g. 15% DMS in Alsever's solution at 2 C for 22 hr (17% fertility) and 10% EG in Alsever's solution at −4.5 C (motility after 16 days was intermediate; the fertilizing capacity after 21 days was 1%).

DISCUSSION

Water coming into the laboratory hatchery is dechlorinated by passage through large granulated charcoal filters. The suitability of this water for incubation of fish eggs was shown in two ways. In the first case the eggs were tested at different times, 2 weeks apart, the same percentage fertility being found in both tests. Secondly, in one series of experiments, eggs were allowed to hatch, producing apparently normal fry. This is taken as indicative of the non-toxic nature of the treated water supply.

The decline (Fig. 1) in fertilizing capacity of sperm on storage, without added protective agents, is qualitatively similar to the findings of others (Barrett, 1951; Foerster, 1965; Withler and Humphreys, 1967; Butcher, 1944; Kubota, 1961). During this experiment, sperm was stored in full, capped vials. Small samples (1 ml) were also stored in 20-ml vials, loosely capped to allow gaseous diffusion between the atmosphere and the interior of the vial. These samples did not show a reduction of fertilizing capacity until after 5 days. Hence, the decline shown in the graph could be due either to accumulation of carbon dioxide and contingent fall in *p*H, or to depletion of oxygen. In general, the *p*H in the full vials fell during storage from 7.5 or above to 7.2 or below. The important effect of the carbon dioxide content of a sperm suspension has been shown for bull (Van Demark et al., 1965). The value of a suitable air space in the storage vial was shown for fowl sperm (Proudfoot and Stewart, 1967), when the optimum air space:semen ratio was in the order of 16:1.

The small rise in percentage fertilization after 4 days storage may be attributable to the fresh eggs used. Although this is suggestive that the decline in general may be due to egg deterioration, this is ruled out by the fact that 5-day-old eggs were successfully fertilized by fresh sperm.

The slightly lower percentage of eggs fertilized at the start in the first (Nov. 7th) series could be due to a possibly more vigorous transportation of the fertilized eggs from the hatchery to the laboratory. The deleterious effect of mechanical agitation on fertilized eggs of pink (Smirnov, 1954) and chum (Smirnov, 1955) salmon has been demonstrated, although it has been shown that there is little loss of fertilized cut-throat trout eggs after 8 hr non-specific agitation by pack-horse (Barrett, 1951).

The addition of unbuffered DMS to sperm lowers the *p*H to 5.7 and destroys its fertilizing capacity. Similarly, unbuffered DMS was found unsuitable for storage of trout and salmon erythrocytes (Hodgins and Ridgway, 1964a). Survival after freezing has been reported for chinook salmon sperm in buffered DMS (Hodgins and Ridgway, 1964b) and for carp sperm in 6–12% glycerine in Ringer's solution (Sneed and Clemens, 1956). Freezing of trout sperm in a variety of glycerinated buffers terminated motility in all samples (Mitchum, 1963). In none of these cases, however, were fertility tests carried

out, motility being the criterion for survival. Sperm from herring testis frozen in 12.5% glycerine in seawater gave 80% fertility after 6 months (Blaxter, 1953). Our attempts to repeat Hodgins and Ridgway's experiment using Atlantic salmon sperm with fast and slow freezing at *p*H 7.5 and 8.2 failed; suspension of sperm in the medium and non-frozen storage at 2 C for 24 hr was not itself injurious (94% fertility).

In modifying the Cortland medium, the potassium level was raised and sodium lowered so that the K^+:Na^+ ratio became 2:1, yet the overall molarity of the medium remained the same. Loss of potassium from cells on dilution has been reported for ram and bull (Quinn et al., 1966). The potassium level was raised here in an attempt to limit a similar loss from salmon sperm.

As in Alsever's solution at 2 C, 12.5% DMS was found to be non-toxic to sperm in the modified Cortland medium and, to a lesser extent, in egg-citrate. Attempts to conserve sperm by use of a 10-fold dilution were unsuccessful.

Pautard's medium has been used to stabilize the flagellae of the spermatozoa of a number of species of marine and freshwater fish, including the brown trout, *Salmo fario*, and Atlantic cod, *Gadus morhua* (Pautard, 1962). It was used here in hope that its protective properties would persist in conditions for preservation. However, little success was achieved.

Little success was also obtained with EG until the longer equilibration time (2 hr) was used. Ultimately, 5% EG in modified Cortland medium allowed storage for at least 38 days at −3 C. In this, the longest storage period tested, as in all 1966 experiments, the time allowed for cells to equilibrate with the diluting solution was ca. 2 hr. High fertility (70%) was obtained under these conditions; 5% EG in Alsever's solution under the same conditions gave 37% fertility. Use of DMS also allowed good storage in Cortland medium but not in Alsever's solution. At −4.5 C sperm stored for 28 days in 5% DMS in modified Cortland medium had 81% fertility. Increasing the concentration of protective agent at lower temperatures did not appear helpful at the concentration tried.

The complete failure of experiments involving glycerol defy explanation; in this connection it may be mentioned that glycerol has been found inferior to ethylene glycol as a protective agent (Fox and Burdick, 1963) and toxic (Polge et al., 1949) to rabbit sperm.

The lack of successful freezing may be due to irreversible damage inflicted when the electrolytes of the cell and suspending medium are concentrated as water is removed as ice (Lovelock and Polge, 1954).

Although several tests have been used to detect live sperm, e.g. motility (Hodgins and Ridgway, 1964b; Sneed and Clemens, 1956) and differential staining (Fribourgh, 1966), the importance of determining fertility for this purpose cannot be overemphasized in view of its unequivocal objectivity. The subjective nature of visual examination of motility renders this test suspect as the sole criterion for full physiological capability of sperm. In characterizing fish sperm, motility rating should be supplemented by a more

reliable indicator. In our experiments, highly fertile samples invariably had highly motile, swirling sperm; samples without motility never fertilized any eggs. Intermediate samples did contain some highly motile cells, but the majority were considerably slower or still. The reduced fertility of these samples can be explained in a number of ways. The loss of activity may be due either to a loss of locomotion in which the sperm would be unable to find or penetrate the micropyle, or to a general deterioration in which the capacity of the cell to fertilize is lost. From work with *Echinus* (Gray, 1928), it has been inferred that in a concentrated sperm suspension activity can be "hidden" by excessive crowding of active sperm by an inactive majority (Foerster, 1965). It seems possible that inactive sperm, by sheer weight of numbers, could become attached to the micropyle, thus preventing entry of live sperm. This spatial hindrance would inevitably limit fertilization.

Difficulties are occasionally encountered in breeding stations in which insufficient male fish are available to allow maximum operation. This necessitates transporting fish to make good the deficiency or storing and transporting sperm.

Interspecific mating has long aroused considerable interest. The present length of storage, much greater than has previously been achieved, is sufficient to extend the scope for interspecific breeding. For example, the Atlantic salmon usually matures in November, the steelhead (*Salmo gairdneri*) in February–March. Spawning may be postponed slightly in Atlantic salmon by keeping the fish in cold water beyond their normal stripping time. On the other hand, ripening of the steelhead may be advanced by administration of suitable pituitary hormones. Thus, the length of storage achieved in this investigation may already make it possible to bridge the gap between the maturing seasons of the Atlantic salmon and the steelhead.

ACKNOWLEDGMENTS

The authors wish to thank Mr R. E. H. MacDonald, Resource Development Branch, Maritimes Region, for making the facilities of his department available; Mr J. L. MacIntosh, Cobequid Fish Culture Station, Collingwood, N.S., for supplying fish; C. G. Hickman, Central Experimental Farm, Ottawa, Ont., for advice on techniques; and Dr D. G. Moore, N.B. Central Artificial Breeding Co-operative Ltd., Fredericton, N.B., for freezing samples in initial stages of the experiments.

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Retinomotor Rhythms in the Bluegill, *Lepomis macrochirus*[1,2]

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ABSTRACT

Bluegills, *Lepomis macrochirus*, entrained to natural or artificial light–dark cycles, were confined to constant darkness for varying periods up to 42 hr at temperatures in the range 20–25 C. The retinomotor activities were determined from histological preparations. In the first night, the eyes became maximally dark-adapted within 1 or 2 hr. The pigment was contracted to a dense basal band, twin cones extended to the boundary of the pigment, and single cones extended into the pigment. After 2 or 3 hr in darkness, these components began a return toward their light-adapted positions, slowly for 5 or 6 hr, and then rapidly during the last 3 or 4 hr, and came to rest in an intermediate state of adaptation at subjective sunrise. In continuous darkness, the retina exhibited a rudimentary free-running rhythm, and there was some evidence of a 12-hr oscillation within the 24-hr period. The amplitudes of the rhythms ranged in decreasing order from twin cones to single cones to pigment and the extreme amplitudes occurred about 2 hr after sunset and at subjective sunrise. Single cones were always more extended than twin cones and only the rod-shaped particles, not the spheres, of pigment appeared to cycle. By the end of the experiment, the rhythms of cones were slightly damped and the rhythm of the pigment was greatly damped. Rods were not studied.

INTRODUCTION

There have been few investigations of free-running retinomotor rhythms in fishes. They have been reported in *Ictalurus* (*Ameiurus*) *nebulosus* by Welsh and Osborn (1937) and Arey and Mundt (1941); in *Astyanax mexicanus* by John and Haut (1964); and in *Carassius auratus* by John et al. (1967). Welsh and Osborn (1937) had studied other fishes and stated that the rhythms must be widespread, but they did not give details of the work or the identity of the fishes, and so the generalization is of unknown significance. Ali (1959) found no persistent rhythms in juvenile *Onchorhynchus* spp., but suggested (Ali, 1961) that in *Salmo salar* the pigment showed a rhythm over the first 24 hr of a 96-hr dark period. However, this rhythm was not synchronized with the diel cycle. Thus, retinomotor rhythms have been described in three species representing three families of fishes, Ictaluridae, Cyprinidae, and Characidae, and appear to be lacking in the Salmonidae. This is, however, a meagre representation of teleost fishes, and is, as stated by John et al. (1967) an insufficient basis for any meaningful generalization.

We selected *Lepomis macrochirus* for these studies on retinomotor rhythms because it would require less effort to accomplish our principal objective,

[1]Received for publication July 25, 1967.
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J. Fish. Res. Bd. Canada, 25(2): 373–381, 1968.
Printed in Canada.

which is to find out whether retinomotor rhythms are generally synchronized with behavioral rhythms in fishes as they were in *Astyanax* (John and Haut, 1964). *Lepomis* would require less effort than other species because a locomotory rhythm had already been described by Davis (1962). He noted that dark-adapted fish, upon exposure to light at night, first settled to the substrate before rising to a free-swimming position and that the time-lapse to the free-swimming position varied regularly through the night. The second reason for selecting *Lepomis* rather than some other species for which behavioral rhythms have been reported was that persistent retinomotor rhythms had not been described in a representative of this family, Centrachidae, and our results, whatever they might be, would give greater meaning to ultimate generalizations on the occurrence and significance of these rhythms. Thus, we have been interested in discovering general correlations and not in making retinomotor rhythms a central issue for exhaustive studies. We have not analyzed the behavior of rods because they are much more difficult to work with and would require more distraction of effort than is necessary for accomplishing our purposes. Whether the rods show persistent rhythms or not is a question for another study.

In this paper, a free-running retinomotor rhythm is considered distinct from the rhythm of the first night. A free-running rhythm begins when the entrainment cue ceases, in this case at sunrise. The rhythm of the first night is not free-running because extinction of light at sunset is one of the normal entrainment cues.

MATERIALS AND METHODS

All fish were selected according to size (total length 6.85 $\pm$ 0.23 cm) in order to reduce variations in the dimensions of eyes. In group C, further selection was effected by using fish with corneal diameters in the range 6.0–7.1 mm. The fish were of unknown ages. They were obtained from a stunted population in a pond near Lancaster, Pa.

Group A: Eight fish, entrained to the natural cycle, were brought to the laboratory on June 12, 1966, for a preliminary investigation of their retinal behavior. The results are included in Table I because they illustrate the relative value of very small samples. The fish were transferred to the darkroom at sunset on June 13. Water temperature was 23.5 $\pm$ 3.5 C. Samples of two fish were fixed in Bouin's solution at 12-hr intervals beginning at 2400 hr, June 13. After fixation for 2 hr in darkness, the left eyes were extracted and the corneas and lenses removed. The eyes were dehydrated in an ethyl-alcohol series, cleared in xylene, and embedded in a 1% beeswax–paraffin mixture. Radial sections were cut at 10 μ on the dorso-ventral axis through the optic nerve. One set of slides was stained with Harris haematoxylin and eosin, and a duplicate set was bleached by the potassium permanganate–sodium bisulfite method and stained in 3% ferric chloride and eosin to expose the cones for accurate identification and measurement.

Group B: The same as group A, but consisting of 40 fish (4 samples of 10).

Group C: Fifty fish were brought to the laboratory in November 1965, for the purpose of comparing the states of the retina at subjective sunrise and sunset with the points of midnight and noon. They were maintained in a room receiving some natural light and were thus on the natural schedule of day-length, but some longer bright periods occurred during this time because the room was sometimes used at night for other work. Beginning January 28, 1966, the fish were placed on an artificial cycle for 24 days. The lights came on at 0705 hr and went off at 1920 hr (12 hr 15 min light; 11 hr 45 min dark). Illumination at the surface of the water was about 50 m-c supplied by daylight flourescent lamps. The aquarium, made of "white" polyethylene, was 1 m in diameter and 0.15 m deep. Water temperature was 21 ± 2 C. The fish were fed daily on pellets (Glencoe Mills Inc., Glencoe, Minn.) at irregular hours generally before noon. On February 21, at 1920 hr, the light was turned off permanently and samples of 10 fish were fixed in Bouin's solution at 2400 hr, Feb. 21, and at 0700 hr, 1200 hr, 1900 hr, and 2400 hr, Feb. 22. After 2 hrs in fixative, sectors, approximately 2 mm square, were excised from the ventral portion of the eyes on, or slightly anterior to, the dorso-ventral axis. The remainder of the procedure followed as in group A.

Group D: Twenty-four fish were brought to the laboratory on June 10, 1966, and were placed on an artificial cycle for 35 days. The phase of the cycle was shifted to provide a convenient working schedule. The light came on at 2415 hr and went off at 1230 hr (12 hr 15 min light; 11 hr 45 min dark). On July 15, at 1230 hr, the light was turned off permanently and samples of two fish were fixed in Bouin's solution at hourly intervals from 1230 hr to 2330 hr, and finally, at 2415 hr. Water temperature was 25 ± 3 C. Other conditions were the same as in group C, and the procedures were the same as in group A.

To record states of adaptation of the retinas, we measured the distances, with an ocular micrometer, from the *elm* (external limiting membrane) to the distal ends of the cone ellipsoids, taking 10 measurements on consecutive twin cones and 5 measurements on consecutive single cones in each eye. For the retinal pigment, we made one measurement from the lamina basalis to the distal boundary of the pigment processes. The mean position of the distal boundary was determined subjectively and was generally close to the extremes, but a few particles of pigment beyond the boundary were usually ignored. All measurements were made on the ventral portion of the retina beginning 1.3 mm from the ventral edge of the optic nerve. This was about 25% of the distance from the optic nerve to the ora terminalis. In group C, represented by isolated sectors of retina, measurements were made in the least distorted part of the section. The thickness of the *vcl* (visual cell layer) was measured at the site of the other measurements.

The results are expressed as RP values (distance to cones or pigment boundary × 100/thickness of *vcl*). This method shows cones extending from the *elm* and pigment extending from the lamina basalis rather than cones

contracting and pigment extending or vice versa. The presentation of results as RP values represents a net reduction of the variations shown by measured distances because the positions of cells and pigment in intermediate states of adaptation are positively correlated with the thickness of the *vcl*, but the correlations are not perfect. RP values are least desirable in representing either fully light-adapted or dark-adapted eyes because they introduce unreal variations as a function of the thickness of the *vcl*. The positions of cones and pigment are independent of the thickness of the *vcl* in these states of adaptation. One obvious advantage of RP values is that they eliminate errors in measurements that occur when the plane of the section deviates from the normal; the relative positions remain constant.

In most of the sections, the tissues were intact and undistorted in the region of the measurements, but in some specimens, the pigment layer was detached and separated from the rest of the *vcl*, or sometimes thrust into a partial overlay of the remaining *vcl*. Delamination of the *vcl* seldom occurred in light-adapted eyes and commonly occurred in dark-adapted eyes. In several specimens, the retina became highly folded in fixation. This folding occurred least frequently in eyes at 2400 hr of the first night and more frequently in the later collections. The pigment in the troughs of the folds was generally compact with a smooth boundary, and in the crests of the folds was more extended with an irregular boundary. We do not know whether the folding caused these differences or whether the differences were characteristic of the eyes. That folding was the cause seemed more likely because in fully light-adapted or dark-adapted eyes, the pigment layer was quite uniform throughout the retina.

In making measurements, we compensated for deranged tissues, but there are undoubtedly some errors attributable to misjudgements. Eleven of the 122 eyes were too much distorted for use. The corrected samples are given in Table I which also shows the variations of relative values and of the actual thicknesses of the *vcl*. Since the measurements were made 1.3 mm ventral to the optic nerve regardless of the size of the eye, they probably include some misrepresentative variations. Also, in using only a small isolated sector of the retina in group C, there was less control on the distance from the optic nerve. These variables together with the previously described limitations of RP values should stress sufficiently the point that RP values are not a precise index of states of retinal adaptation.

RESULTS

The distribution of twin and single cones, as seen in tangential sections, was predominantly regular with one single cone surrounded by four twin cones at 90° points. Radial sections showed a variety of ratios of the two types depending on the path of the section, but most often showed a pattern of one twin followed by one single as shown in Fig. 1. The pigment throughout the retina consisted of basal spheres and distal rods.

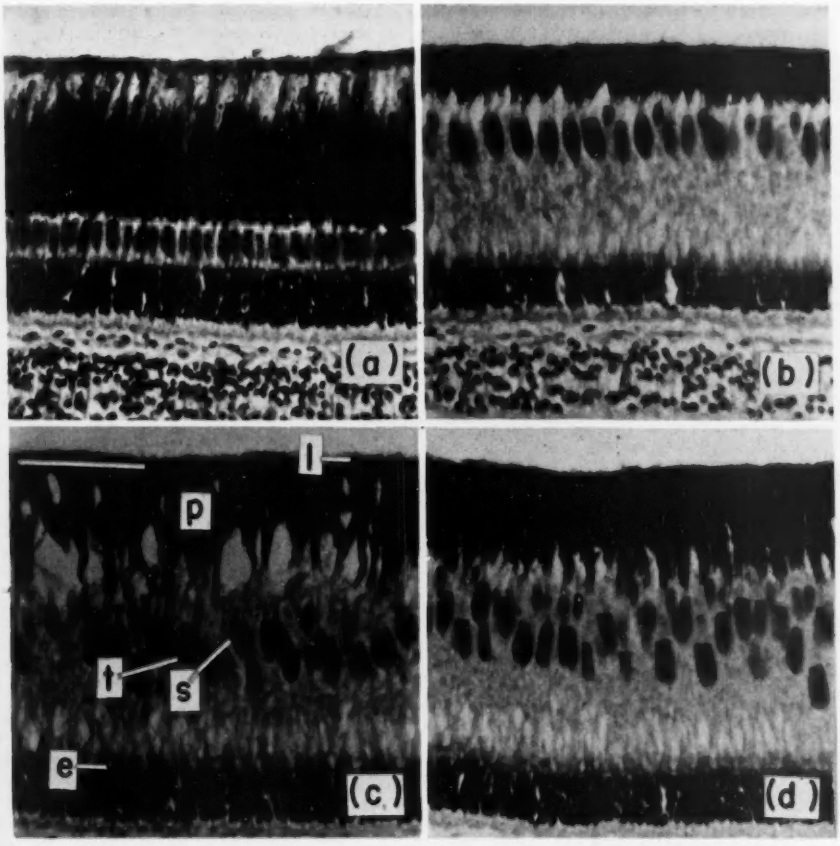

FIG. 1. Photomicrographs of retinas of *Lepomis macrochirus*. (*a*) light-adapted during the day at about 50 m-c; (*b*) dark-adapted 2 hr after lights off, pigment detached; (*c*) dark-adapted at subjective sunrise, pigment detached; (*d*) dark-adapted at noon of first day in darkness. Harris haematoxylin and eosin, 10 μ, 450×; *e*, external limiting membrane; *l*, lamina basalis; *p*, pigment; *s*, single cone; *t*, twin cones, bar equals 50 μ.

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TABLE I. Mean values with one standard deviation of RP values (relative position) of cones and pigment and of thickness, in microns, of the *vcl* (visual cell layer) in eyes of *Lepomis macrochirus* in continuous darkness.

Group	Time (*hr*)	Sample size	RP values			*Vcl* thickness in μ
			Single cones	Twin cones	Pigment	
A	2400	2	72.2±4.4	67.0±0.4	33.8±6.6	88.4±14.2
	1200	"	62.9±0.3	46.9±1.4	35.8±4.3	86.6±8.4
	2400	"	70.6±5.6	62.2±6.1	30.9±0.5	104.3±4.2
	1200	"	63.9±2.7	48.1±1.5	41.3±5.5	97.2±15.8
B	2400	9	68.3±2.9	61.0±3.0	30.2±5.0	109.5±14.1
	1200	"	60.3±4.8	45.3±5.7	39.3±6.6	117.9±17.7
	2400	"	70.4±3.6	60.5±5.9	33.7±4.2	103.8±6.8
	1200	8	64.7±4.9	49.7±4.3	35.1±4.9	105.0±5.6
C	2400	10	66.3±6.6	62.0±7.1	32.2±6.0	79.3±5.1
	0700	9	45.8±9.5	34.9±7.0	47.4±8.1	91.9±6.5
	1200	"	54.3±6.7	46.0±8.1	47.2±6.3	80.0±8.9
	1900	"	48.3±5.0	40.3±5.7	45.6±6.4	86.2±8.9
	2400	"	61.5±9.1	52.5±10.3	37.4±6.4	83.8±4.0
	Hr in darkness					
D	0	2	22.3±1.3	23.1±0.2	68.5±0.9	81.8±1.8
	1	"	70.7±0.7	66.0±2.9	27.1±0.7	82.9±2.3
	2	"	71.5±5.3	67.2±2.3	24.9±0.5	103.0±11.2
	3	"	69.3±1.6	61.7±3.2	24.3±0.7	119.8±5.6
	5	"	67.9±7.3	60.4±8.8	26.8±2.4	97.4±5.6
	6	"	66.3±7.1	60.5±7.7	33.8±4.9	85.1±15.7
	7	"	66.5±2.1	62.0±4.8	31.1±5.0	91.8±11.2
	8	"	66.4±1.1	58.7±1.7	30.9±1.6	97.4±5.6
	9	"	63.2±10.8	54.0±8.0	33.0±3.7	100.8±9.0
	10	"	57.3±4.9	45.9±3.1	34.7±4.5	116.5±2.2
	10½	"	48.1±1.0	33.4±0.2	42.8±6.2	103.0±11.2

In the light-adapted eye (Fig. 1*a*) the cones were fully contracted at an illumination of 50 m-c, but although the boundary of the pigment was fully advanced, the pigment was not totally extended as is shown by the streamers projecting toward the lamina basalis. In eyes which were light-adapted at about 300 m-c (aquarium stock-room) the pigment was totally withdrawn from the basal one-third of the pigment cells. In either case, the particles formed a dense opaque band through the center of the *vcl*. In the dark-adapted eye (Fig. 1*b*), the pigment was contracted into a dense band at the base of the *vcl*. Twin cones were extended to the boundary of the pigment, and the single cones were extended partially into the pigment.

The rhythms of cones and pigment during the first night in darkness are shown in Fig 2. The RP values at 0 hr represent light-adapted eyes and

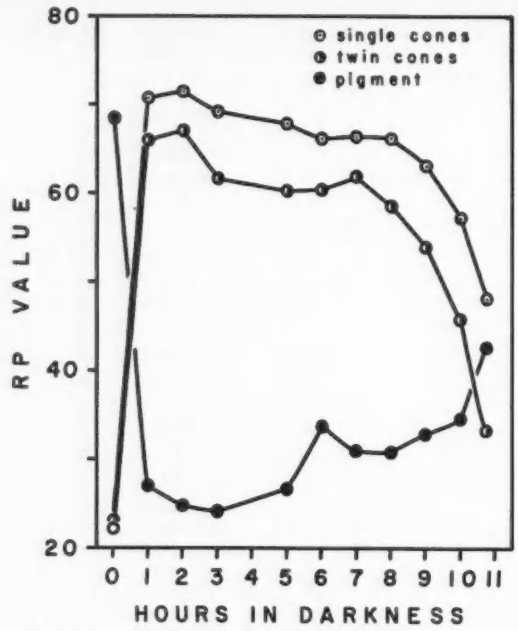

FIG. 2. Rhythms of cones and pigment relative to thickness of visual cell layer in *Lepomis macrochirus* during first night in darkness.

provide a reference for estimating the extreme amplitudes of the rhythms. The two characteristics to note are the general shapes of the curves and the rates of migration of the three components. Dark-adaptation progressed rapidly and was essentially completed within the first hour of darkness though the graphs indicate that completion of the process by the cones required 2 hr and by the pigment, 3 hr. The return toward the light-adapted state began after 2 hr in darkness, possibly 3 hr for the pigment, and the rates of return of the three components were roughly equal up to 6 hr. Thereafter, the twin cones contracted most rapidly and, at subjective sunrise, rested at about 11 RP units from the light-adapted position. Single cones and pigment both rested at about 27 RP units from their light-adapted positions at subjective sunrise, but they arrived there in quite different fashion. The single cones showed a progressive increase in rate of movement whereas the pigment moved slowly until the last hour, when it shifted abruptly. Excluding the changes between 0 and 1 hr, there were no statistically significant differences between consecutive points within the individual plots of twin cones, single cones, or pigment (Student's *t*-test).

The rhythms of cones and pigment persisted in constant darkness (Fig. 3*a* and *b*) but they were rudimentary from the beginning. The retina did

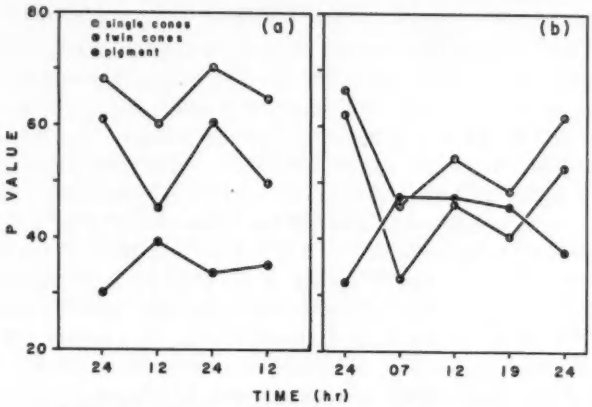

FIG. 3. Rhythms of cones and pigment relative to thickness of visual cell layer in *Lepomis macrochirus*. (*a*) group B, 42 hr constant darkness; (*b*) group C, 29 hr constant darkness; subjective sunrise, 0705 hr; subjective sunset, 1920 hr.

not achieve a full state of light-adaptation in the first 1200-hr samples. The rhythms became increasingly rudimentary as the experiment progressed as a result of progressive damping. The weakest rhythm was shown by the pigment and this rhythm was greatly damped by the end of the experiment. Both Fig. 3*a* and *b* indicate that damping of the rhythm in the pigment began by the second 2400-hr sample, but the evidence on damping, or at least initiation of damping, in the rhythms of the cones was divided. Figure 3*a* shows only slight damping of the twin cones in the second 2400-hr sample but Fig. 3*b* shows obvious damping of both single and twin cones in the second 2400-hr sample. Damping of the rhythms of single and twin cones in Fig. 3*a* (group B) was not well marked until the last 1200-hr sample.

The extreme amplitude of the light-adapted phase of the rhythms occurred at subjective sunrise, 0700 hr (Fig. 3*b*). There was a secondary extreme at subjective sunset, 1900 hr, for the cones, but no change occurred in the pigment through the subjective day. The secondary return of cones toward the dark-adapted state at 1200 hr was not significantly different from the values at 0700 hr and 1900 hr.

In all groups that were confined to prolonged darkness, the mean histological appearance of the pigment changed with time. The profile of the distal border ranged from smooth to slightly irregular (Fig. 1*b*) to irregular (Fig. 1*d*) to highly irregular (Fig. 1*c*) as determined by the lengths of extended processes. At 2400 hr on the first night, the border varied from smooth to irregular, and at 1200 hr on the first day, it was highly irregular. At 2400 hr on the second night, it varied between irregular and highly irregular, and at 1200 hr on the second day, it was irregular. It appeared that the damping of the rhythm of the pigment involved a trend toward irregularity.

DISCUSSION

Although it is clear that the retinomotor rhythm of the first night progressed at first very rapidly, then slowly, and then again rapidly (Fig. 2), it is equally clear that the small fluctuations in the curves are not necessarily correct representations of the rhythms for the species. The RP values at 0 and 1 hr, representing fully light-adapted eyes and fully dark-adapted eyes respectively, might well have differed by several RP units by chance. If the eyes of the fourth sample with the *vcl* thickness of 120 μ (Table I) had represented the first sample, which had a thinner *vcl*, the RP value for single cones would have been about 15 rather than 22. It must be assumed that the other points in the curves are equally imprecise. One cannot determine from Fig. 2 the exact hour of maximal dark-adaptation or whether the troughs in the curves of cones between 2 and 7 hr represent a valid trend. One deviant sample would have obliterated this apparent trend. It seems quite likely that the peak in the pigment at 6 hr represents an unusually deviant sample (see Table I).

Although the secondary peaks in the rhythms of the cones at 1200 hr (Fig. 3*b*) were not significantly different from the values at 0700 hr and 1900 hr, they probably are a correct representation of the retinal behavior because they were based on larger samples that had been more carefully selected for uniformity of ocular dimensions than were the values in Fig. 2. As such, these peaks suggest a 12-hr oscillation superimposed upon the 24-hr rhythm.

Another conclusion which seems certain is that the extreme amplitudes of the persistent rhythms were underestimated by sampling the fish at 1200 hr and 2400 hr. The curves for cones (Fig. 3*a*) would have been noticeably amplified if samples had been taken 1 or 2 hr after sunset and again at subjective sunrise. Figure 2 supports Fig. 3*b* in showing that the eyes did most nearly approach the light-adapted condition at subjective sunrise. It is possible that a curve based upon hourly samples would fix the extreme value at some time between subjective sunrise and noon.

The disagreement between Fig. 3*a* and *b* regarding the damping of the rhythms of the cones in the second 2400-hr samples might be the result, again, of unreal variations induced by conversion of the results of different-sized eyes to RP values. (Compare thicknesses of the *vcl* of groups B and C in Table I.) Since group C was less variable by virtue of the selection for standard ocular dimensions, it should be a better representation than group B. Another difference that might contribute to the disagreement is that group C was collected in November 1965, and group B in June 1966, and there could be seasonal influences. A final difference was the temperatures in the laboratory: 23.5 $\pm$ 3.5 C for group B and 21 $\pm$ 2 C for group C. Arey (1916) showed that the migration of retinal components is clearly altered by different temperatures though the effective differences in his studies were much greater than the differences in temperatures between groups B and C. The question here is whether different temperatures might affect the amplitudes of presistent rhythms.

The curves in Fig. 2, if pigment were expressed as percentage of contraction rather than percentage of extension, would correspond closely with the behavioral curve of Davis (1962). He found that the time-lapse between application of lights and attainment of a free-swimming posture by *Lepomis* rose rapidly after sunset and then declined through the night, and rested at about 50% of the maximum just before sunrise. The value of 50% agrees well with the positions of single cones and pigment at subjective sunrise though less closely with that of the twin cones (Fig. 2).

It would not be surprising to find that behavioral rhythms and retinomotor rhythms are commonly correlated in fishes, but much work remains to be done before the significance of these correlations is understood.

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Infectious Pancreatic Necrosis: Selection of Virus-Free Stock from a Population of Carrier Trout[1]

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ABSTRACT

Infectious pancreatic necrosis (IPN) is a virulent disease of young trouts and is easily transmitted from infected animals through water and with eggs. At present, the most effective control measure consists of propagation of specific pathogen-free stock. Methods are described for using fish cell cultures to detect IPN virus in peritoneal washes, feces, and gonadal fluids and thereby to identifiy carrier and non-carrier fish. The procedures were used to derive virus-free progeny from a hatchery trout population containing about one-third carrier fish. The progeny were reared to maturity, spawned for 3 successive years, and at each spawning they proved free of virus. Recommendations and precautions are given for those who may wish to apply the procedures.

INTRODUCTION

Infectious pancreatic necrosis (IPN) is a viral infection of trouts that usually occurs as an acute disease producing high mortality in very young hatchery species. Although little is known about IPN in the wild, the virus could very well have a similar effect in natural populations of trout. As the hosts become older their resistance increases in some as yet unknown way; mortality among fingerlings is usually less than among fry, and adults are apparently refractory. In IPN as with most infectious diseases, there are survivors. To all outward appearances, fish which survive IPN are normal, but they nevertheless have an active infection and shed virus for much and possibly all of their lives. This is a fairly common phenomenon in virology, and animals which have such infections are termed *carriers*.

Carrier fish shed virus — often as much as several million infective virions per milliliter of feces, seminal fluid, and in the so-called ovarian fluid. Carrier fish thus provide excellent distribution of the virus and help insure its long-term survival. This is especially true in the transmission with eggs and sperm, because this provides continuity between host generations. Once an epizootic has been initiated, the victims also shed large amounts of virus through the water, and new hosts become infected probably by ingesting the virus with food and possibly through the gills.

IPN is probably the best known viral disease of fish, and there is a growing body of literature on the subject. Wolf has recently reviewed the disease (1966a), and in a more comprehensive work, the virus (1966b).

[1]Received for publication November 6, 1967.

J. Fish. Res. Bd. Canada, 25(2): 383–391, 1968.
Printed in Canada.

Viral diseases of animals are controlled by ecological manipulation, therapy, immunization, and avoidance, but only the latter is presently effective with IPN. As presently known, the ecology of IPN shows no aspect which would be vulnerable to practical methods of intervention. Today, therapy of infection by true viruses is still restricted to special circumstances with a very few diseases of man and other warm-blooded vertebrates. Immunologic procedures are in development for IPN, but the margin of value of IPN vaccine may be reduced by the very young age of the usual victims.

We cannot ignore the fact that contaminated eggs and/or water make it virtually impossible for some hatcheries to avoid IPN. Although it is not a control measure, extra eggs are sometimes hatched to compensate for anticipated losses. In other cases, virus-free water which may be available, but in limited quantity, can be used for incubation and rearing to delay as long as possible the first exposure, and hence to reduce the percentage mortality.

The most effective control of INP, avoidance or prevention, is currently accomplished by propagation of virus-free stock in an uncontaminated water supply, but stocks of brood fish which are now known to be free of IPN virus are too few to meet existing demands for eggs. Though they do carry the virus, certain strains of trout are unique or have desirable heritable characteristics. According to Snieszko (1957), the Bellefonte, Pennsylvania, stock of brook trout was one of two strains found by Wolf (1954) to be particularly resistant to furunculosis and ulcer disease. This was subsequently confirmed by Snieszko et al. (1959). Desirable though it was from the standpoint of resistance to severe bacterial infections, the Bellefonte, Pa., strain of brook trout was circumstantially implicated as a source of IPN (Snieszko et al., 1957). Subsequently, virus — serologically identified as IPN virus — was readily isolated from adult females in the population of brood fish at the hatchery (Wolf et al., 1963). In a second sampling, virus was isolated from 3 of 15 female fish and from 2 of 14 males. All agents were serially transferred to a dilution equivalent to 10^{-50} original material then serologically identified as IPN virus (unpublished research). The purpose of the work reported here was to test a sample of Bellefonte brook trout brood stock and to determine which were carriers and which were free of IPN virus. Secondly, it was planned to spawn a group of carrier fish in order to determine the outcome, and to spawn a group of healthy parents in the hope of deriving IPN-free progeny.

MATERIALS AND METHODS

Trout

Thirty-one 3-year-old Bellefonte, Pa., strain fish were provided through the courtesy of A. D. Bradford of the Pennsylvania Fish Commission from stock maintained at the Fisherman's Paradise Hatchery. The fish were individually identified by tagging with numbered metal jaw tags and transported to the Eastern Fish Disease Laboratory. There they were fed commercial trout pellets and kept in water whose physical and chemical properties have been described elsewhere (Warren, 1963).

FISH PROPAGATION AND SANITATION

We share with the Leetown National Fish Hatchery a spring which has been tiled, covered with rock, then soil, and has an outlet which has been effectively screened and housed to exclude fish. The water has a long history of being free of fish viruses; in addition it is virtually free of all fish pathogens except the ubiquitous myxobacteria and motile pseudomonads and aeromonads. Our work was of necessity conducted indoors but near tanks and troughs of fish which often were used for other disease research. Contagion was not spread and hygienic isolation was maintained by simple but disciplined procedures. Separate feeding and cleaning equipment was used, and feed containers were covered to exclude flies and other animals which might spread infection. Nets, pans, other equipment, hands and arms were routinely disinfected in a solution of 1:1000 Roccal^{R} prior to use with any of the fish or eggs.

Fish were paired for spawning on the basis of preliminary viral testing (to be described). Both parents in one group were provisionally free of IPN, but the second group consisted of matings wherein one or both parents were known to be carriers. Spawning and egg incubation were conducted most carefully because a single undetected carrier could infect healthy progeny and defeat the purpose of the work. Fish were anesthetized with a branchial spray of 1:5000 tricaine methanesulfonate then blotted dry. Eggs from individual females were stripped into sterile gauze-covered paper cups for draining; they were then transferred to disinfected porcelain pans where they were fertilized with sperm from a male which had been similarly prepared. After fertilization, the eggs were hardened and transferred to individual quart-size plastic jars for incubation and rearing. The jar incubators were developed by Buss and Waite (1961) and kindly provided by them for use in our work. Between the spawning of each pair of original Bellefonte fish, the operators' hands and arms, and all pertinent equipment was disinfected. From eggs through testing as advanced fry, the offspring of each pairing were maintained as a separate lot, and until the results of all the testing showed otherwise, each lot was handled as though it were infected. Hatching and early feeding occurred in the plastic jars, then the fry were transferred to glass Downing jars and later as their size demanded, to tanks.

FISH CELL CULTURES

RTG-2 cells, an established fibroblastic line of rainbow trout (*Salmo gairdneri*) gonadal tissue origin (Wolf and Quimby, 1962) were grown in static 16 × 125-mm culture tubes at 20–23 C. Tubes were seeded with 1 ml of cell suspension. Growth medium consisted of 93% Eagle's Minimal Essential Medium plus 7% fetal bovine serum; 100 units of penicillin; 100 μcg streptomycin; and 25 units nystatin per ml. Cultures were inoculated with test material 2–4 days after seeding at which time the cell sheet was confluent or nearly so.

Virus Detection

Procedures used to detect IPN virus consisted of several steps. Feces and peritoneal washes were each tested prior to spawning. Another test was made of eggs and sperm at spawning, and the final testing was for virus in the progeny.

PERITONEAL WASHES

Using aseptic technic and sterile equipment for each animal, individual fish were injected intraperitoneally with 5 ml complete physiological phosphate buffered saline at *p*H 7.3 (PBS).[2] The body of the fish was massaged gently to wash the saline over the visceral organs and hopefully to pick up virus from pancreatic tissue which is scattered midst the pyloric caeca. PBS was then aspirated and 0.1–0.3 ml inoculated directly into one or more cell cultures. Cultures were incubated for 7 days then examined for cytopathic effects (CPE). Cell sheets which showed the slightest CPE were suspect, and 0.1 ml of the medium was transferred to a new culture. If the second culture developed CPE within a day or so, the results were considered presumptive evidence for virus and the appropriate fish considered a carrier.

FECES

Measured samples of feces were expressed from anesthetized fish and suspended in 9 volumes of PBS. Gross particulate matter was allowed to settle after which a bacteria-free filtrate was prepared by passing the supernate through a Millipore microfiber pre-filter and an HA (0.45 μ) membrane. Inoculant volume of fecal extract was only 0.1 ml per culture tube because larger volumes resulted in a greater incidence of CPE, and additional testing was necessary in order to distinguish toxic CPE from viral CPE. Virus causes CPE that can be transferred from culture to culture, but because of dilution, CPE that results from toxicity is lost during transfer. Determining the cause of CPE was especially important in testing feces, and in some cases two transfers were required.

Identification of IPN Virus

Cell culture neutralization test was used for serological identification of IPN virus. Reference antiserum prepared in rabbits had a neutralizing antibody titer of at least 1:8000 against about 10^3 tissue cell culture infectious doses — 50% end point ($TCID_{50}$). The quantitation used was generally adequate for the amount of IPN virus in either killed RTG-2 cell cultures or trout fry.

EGGS AND SPERM

Fluid which drained from the unfertilized eggs at stripping was collected from the cup, and 0.1 ml was used without filtration for direct inoculation

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[2]We have subsequently determined that Hanks' and Earle's salt solutions which are available commercially are equally satisfactory, and in an emergency Roger P. Dexter, Fishery Biologist, Craig Brook NFH, Maine, has used isotonic sodium chloride solution which he obtained from a hospital.

of two culture tubes of cells. Males were stripped into tubes, the bulk of the sperm being used for fertilization and, where possible, a portion was tested in cell culture. Because the total volume of semen was small, the inoculant volume did not exceed 0.1 ml per culture.

PROGENY

Fry from each mating were first tested for virus at 1–2 months of age. Where symptoms of IPN were observed, affected animals were selected. In other lots, the weak or dead were tested, but some lots appeared completely normal and a random sample was chosen. Fry were homogenized in PSB, diluted to 10^{-1} or 10^{-2}, filtered through an HA membrane, and inoculated into cell cultures.

Additional virological testing was made of IPN-free progeny at the 1st, 2nd, and 3rd year of their life. Trout were spawned according to normal hatchery production methods. At each of these spawnings, pooled fluid from each batch of fertilized eggs was aspirated, clarified by centrifugation, filtered, and inoculated into cell cultures.

PROTOCOL

We used a Millipore HA membrane filtrate prepared from a 10^{-3} dilution of affected cell culture medium or from whole victim fry (fresh, preserved at −20 C or lower, or preserved in 50% glycerol at 4 C) homogenized and diluted in PBS to 10^{-3}. One volume of diluted material containing the suspect IPN virus (usually 0.3 ml) was mixed with an equal volume of normal rabbit serum diluted 1:100. Rabbit anti-IPN serum diluted 1:100 was similarly mixed with another volume of suspect material and the mixtures allowed to react for 1 hr at 15–25 C. (We have subsequently determined that 10 min is sufficient.) Duplicate confluent tubes of RTG-2 cells were each inoculated with 0.2 ml of the normal serum/virus mixture, and another two tubes inoculated with the anti-IPN serum/virus mixture. Uninoculated control tubes were used and all were incubated at 15–23 C until CPE was evident.

INTERPRETATION

If the original material contains IPN virus, the cultures receiving normal serum lacking the specific IPN antibody will show CPE in 18–48 hr depending upon the amount of virus and the temperature of incubation. Antibody in the reference serum will prevent infection by IPN virus; therefore, the cells will appear normal and indistinguishable from the controls. Other salmonid viruses will produce CPE in all but the uninoculated control cultures.

RESULTS

Furunculosis, a bacterial infection known to be enzootic in the source lot of fish, erupted shortly after the experimental work began, presumably a response to the stress of tagging, transporting, and handling fish which were nearly ready to spawn. All fish received an intraperitoneal injection of 15 mg chloramphenicol succinate. Depending upon the weight of the fish, this was

a dosage of from 2 to 5 mg per 100 g body weight. The disease was brought under control, but 12 of the fish were lost. There were, to the best of our knowledge, no other complicating infections.

On a day when most of the fish were considered ripe, 12 fish were used for 7 matings. There were five pairs which were tentatively considered virus-free because the testing of feces and peritoneal washes had been negative. The test at spawning, however, revealed a previously undetected carrier — #8442 (Table I). Subsequently, virus was twice isolated from dead progeny fry from this female. Two matings were made using carriers which had been detected by testing peritoneal washes and/or feces. Clinical IPN disease developed in the progeny of one, and virus was isolated from dead and dying fry. All virus isolated from fry and from fish actually used for spawning was serially passaged in cell culture to an equivalent of 10^{-50} original material, then serologically tested by serum neutralization and unequivocally shown to be IPN virus.

Two lots of healthy progeny were reared indoors without complications. Occasional fingerlings died, but all tests for virus were negative, and the two lots were ultimately combined. Some of the fish matured at 1 year of age and they were spawned. Samples of each of the pools of eggs and sperm were tested and found to be negative for virus. The tests were repeated at the 2nd and 3rd year, and each time the fish proved to be IPN-free.

TABLE I. Summary of virological testing of a sample of a brood stock from an IPN carrier population.

Sex	Tag No.	Test before spawning	Test at spawning	Test of fry	Remarks
Group I: Feces and/or peritoneal washes showed no virus prior to spawning					
♂	8499	–[a]	0	Free of IPN	Good hatch
♀	8482	–	–		
♂	8494	–	–	0	No hatch
♀	8493	–	–	0	
♂	8464	–	–	0	No hatch
♀	8448	–	–	0	
♂	8472	–	–	Free of IPN	Good hatch
♀	8452	–	0		
♂	8464	–	–	IPN virus	Discarded
♀	8442	–	+	isolated	
Group II: Feces and/or peritoneal washes showed one or both parents to be IPN carriers					
♂	8494	–	–	IPN virus	Clinical IPN
♀	8450	+	+	isolated	developed
♂	8461	+	+	IPN virus	Poor hatch
♀	8459	+	+	not ioslated	

[a] – = negative; + = positive; 0 = test material not available.

Using the combined results of testing feces peritoneal washes and either eggs or sperm, a carrier incidence of about one of every three fish is indicated; this is very close to the incidence of 12 carriers among 41 fish tested at spawning during earlier work with this population. It appears from these limited data that sex products are most accurate in reflecting the IPN carrier state. Conversely, these and other data show the peritoneal wash to be least sensitive in detecting virus.

Supplemental results from comparative field tests are relevant. In 1965, we used peritoneal washes pooled from five fish for testing approximately 10% of a brood stock population of 2900 rainbow trout (*Salmo gairdneri*). Virus was found in one sample, and it was identified by cell culture neutralization test to be IPN virus. The incidence in the population was surprisingly low. Assuming that the pooled sample represented at least one carrier fish, the incidence in the population was at least three-tenths of 1%. The lot of fish represented a splendid opportunity for comparative testing, and we made a second survey 3 months after the first. We took paired samples of feces and of peritoneal washes from 100 five-fish pools, and inoculated duplicate cell cultures with bacteria-free filtrates of each. A pool was considered positive for virus only if CPE was produced and shown to be transferrable by carrying it through six serial cell culture passages — a procedure which effected a cumulative dilution exceeding 10^{-6} of the original material. Virus was found only in the peritoneal wash in 3 cases, in both peritoneal wash and feces in 5 cases, and only in feces in 24 cases. Thus, the examination of feces revealed over 90% of the known carriers whereas peritoneal washes showed only 25%. The use of feces for virological testing is both economical and simple, and although it may not be as sensitive as the use of eggs and sperm, it has no seasonal restrictions.

RECOMMENDATIONS

The results show that a series of virological tests could be used to distinguish between virus-free and carrier trout, and that the information could then be used to mate IPN-free fish and thereby derive progeny which were also free of this disease. The work also shows that IPN virus was transmitted with eggs and possibly with sperm and that it could be isolated only from fry which came from matings in which one or both parents had been identified as a carrier.

Avoidance is presently the most effective and practical measure for controlling IPN, and this measure requires propagation of virus-free stock. The following procedures are recommended for selecting parent fish, breeding them, and thereby establishing virus-free stock from populations of brood fish which carry IPN. It is emphasized that the derived young will still be susceptible to the disease and that such a program should not be undertaken unless the eggs can be hatched and the offspring reared at least into fingerling stage in IPN virus-free water. Wells or appropriately protected springs usually furnish such water, but adequate chemical or radiation treatment of contaminated supplies can give the same end result.

Presumably, justification for starting such a program would be to derive IPN-free stock from strains of fish with desirable hereditary characteristics. It is therefore essential that precautions be taken to maintain the genetic qualities which characterize the particular strain or population of fish. We have sought the advice of Dr J. E. Wright, Jr., Professor of Genetics, Pennsylvania State University, on this subject. He suggests that population heterogeneity will likely be maintained in the small numbers of fish which make virological testing simpler (as low as 25 parents) if random matings are made among fish of diverse size (reflecting different growth rates) and at different times during the spawning season. In essence, one seeks to avoid inbreeding, and uses diversity in spawning time and in size or growth rate to maintain genetic heterogeneity.

The preceding requirements being met, the first step should be tagging or other method of identifying individual fish. Next, those carriers which are easily detected should be eliminated. Because it is both easier and more sensitive than peritoneal washes, virological examination of feces should be used for that purpose. A single test will reveal many carriers, but more will be found if additional testing is done. It is likely, however, that examination of feces alone cannot be depended upon to reveal all carriers; therefore, other infected individuals will be found with the most sensitive test — the examination of eggs and sperm. Of course, one might argue that the procedure could be simplified by using only the spawning test, but it seems prudent to cull as many carriers as possible prior to spawning so that the accidental spread of infection during incubation will be minimized.

At spawning, the fish should be handled as described herein — equipment and hands and arms of personnel should be thoroughly disinfected between each mating. From that point until the fry have fed for several weeks, the progeny of each pair should be kept as a separate sub-lot and isolated from similar sub-lots. If IPN symptoms appear in a sub-lot of fry, one should attempt to isolate the virus. If the fry prove to be infected they should be carefully removed from the jar and destroyed so as to kill the virus. The work should not be considered complete until a virological test is made of each sub-lot of fry. Dead, dying, or weak specimens should be used if available, since IPN may be the cause of the condition, but if such are not available, a sample of normal fry should be made several weeks after the start of feeding. By waiting, the virus, if it is present, will have an opportunity to infect more fry in the sub-lot and thus make its detection easier. Sub-lots of fry which prove virus-free and which were derived from parents which prove negative in all tests may be combined to form the new population.

To develop an IPN-free stock of trout from a carrier population requires a considerable expenditure of time and effort, and one should keep that fact in mind and provide protection measures appropriate for maintaining the fish virus-free. Some of the more obvious measures include an embargo on fish and eggs from other hatcheries unless one can be positive that they do

not carry the virus — a difficult feat. Similarly, trucks, equipment, and personnel from other hatcheries should be prohibited, or if that is not practical, admitted only after thorough disinfection. Such a measure was effectively initiated several years ago by Manager Harold Fisher of the Paint Bank National Fish Hatchery, Virginia. It may even be necessary to restrict visitors, and although this may sound like an extreme measure to fish-culturists, such measures have been in effect in that portion of the poultry industry that is devoted to production of fertile eggs.

Inefficient business does not survive in today's competitive world. The propagation of diseased fish is a form of inefficient production that will not endure in the commercial field and it should not be tolerated in conservation agencies.

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Effects of Light on Schooling and Feeding of Jack Mackerel, *Trachurus symmetricus*[1]

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ABSTRACT

Schools of six jack mackerel each were photographed with infrared film at eight levels of luminance and also in darkness. Three indices were used to measure the behavior of the school from motion pictures. Two of the indices, mean distance to nearest neighbor and mean separation distance, were measures of the distances between individuals in a school; the other, mean angular deviation, was a measure of differences in orientation between individuals. A value for each index was calculated for each motion picture frame.

From 12.1 to 6×10^{-6} ft-L no differences existed in the angular deviation of the school or in the distances between fish. At 6×10^{-7} ft-L the intervals between fish were much larger than at higher levels of brightness and groups showed little uniformity in their orientation. Below 6×10^{-7} ft-L (darkness) schools were dispersed and the distributions of values of angular deviation were random.

The ability of jack mackerel to feed on live adult *Artemia* was also tested at eight levels of luminance and in darkness. The number of *Artemia* eaten at 6×10^{-5} ft-L was about half of that eaten at the normal daytime level of 12.1 ft-L. Few *Artemia* were eaten at 6×10^{-7} ft-L and none in darkness.

Comparison of these data with measurements of light in the sea indicated that jack mackerel probably would be able to maintain schools near the surface on a moonless starlit night and that they probably could feed effectively near the surface on a full moonlight night.

INTRODUCTION

The dominant modality involved in the formation and maintenance of fish schools is vision (Atz, 1953; Breder, 1959). All species studied at the time of Atz's (1953) review of schooling behavior were reported to be unable to maintain a school in darkness. No measurements of illumination were made in these studies; thus, the level of illumination at which schooling ceases is of interest and has been the object of a number of investigations (see reviews by Shaw, 1961 and Blaxter, 1964). A wide range of visual thresholds for schooling exists from 5×10^{-2} ft-c for *Menidia* (Shaw, 1961) to 1×10^{-6} ft-c for *Astyanax* (John, 1964). John (1964) pointed out that the reported variations in visual threshold for schooling probably are as much the results of arbitrary definitions of schooling and photometric errors as they are variations among species.

In this paper I have related the schooling behavior of juvenile jack mackerel, *Trachurus symmetricus* (Ayres), to the photometric brightness or luminance in an experimental tank. I have also included data on the effect of different levels of luminance on feeding behavior to provide additional information on the relation between light and the behavior of jack mackerel.

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[1]Received for publication August 30, 1967.

J. Fish. Res. Bd. Canada, 25(2): 393–407, 1968.
Printed in Canada.

To avoid as many photometric errors as possible, room, tank, and light sources were designed to provide a diffuse illumination and a field of uniform luminance. Luminance in the tank was changed without altering the spectral composition of the light and photometric measurements were made within the tank.

APPARATUS AND GENERAL METHODS

The facilities consisted of a concrete block room constructed on a foundation separate from the rest of the experimental aquarium at the Fishery-Oceanography Center, La Jolla, Calif. The room was light-tight and was supplied with fresh circulating seawater. The floor, walls, ceiling, and all structures in the room were painted with flat white paint of high reflectivity (photometric sphere paint). The lens of a 16-mm time-lapse movie camera was placed in a port in the ceiling of the room above a plexiglass tank, 183 × 244 × 52 cm, constructed in the same relative dimensions as a 16-mm motion picture frame (Fig. 1A and B). The inside surfaces of the bottom and the sides of the tank were sprayed up to the waterline (18 cm) with a white acrylic paint. A rectangle on the bottom of the tank (61 × 49 cm) was left unpainted so that photometric measurements could be made from beneath the tank. During an experiment this transparent window was covered by a piece of white plexiglass of about the same transmittance as the painted bottom.

The room was illuminated by 10 fixtures (Fig. 1C) spaced at regular intervals around the perimeter of the tank. Each fixture supported five No. 81, 6.5-v, 2885 Kelvin lamps; four of the lamps were attached to the upper ends of plungers which were inserted inside tubes lined with black velour casket cloth; and one lamp was fixed and not enclosed in a tube. A bushing which contained a disk of white plexiglass and often a neutral density filter was inserted into the end of each of the tubes. The upper ends of the tubes were enclosed by a globe of white opal glass.

Only 10 lamps (1 per fixture) were on at any one time; different lamps in the fixture were used to produce different levels of luminance within the tank. The output of each adjustable lamp was changed by moving the plunger and by changing neutral density filters. The fixed, non-adjustable lamps produced the normal day luminance; the adjustable lamps produced lower test levels of luminance. Variation in luminance among lamps did not exceed 10%.

Kodak high-speed infrared film was used to photograph the fish. Infrared radiation was obtained from three 250-w, 3400 Kelvin quartz iodine lamps (Fig. 1D) suspended beneath the tank facing the floor. Mounted under each lamp was a water bath and a Corning 7-69 filter which transmitted infrared radiation and absorbed visible radiation. This system provided adequate infrared photographs of fish schools. Figure 2 shows that sources of infrared radiation much below 3400 K would be inefficient and that photography would be difficult in water deeper than 50 cm. Infrared photography of fish and other aquatic organisms has been discussed in detail by Duncan (1956) and Baylor (1959).

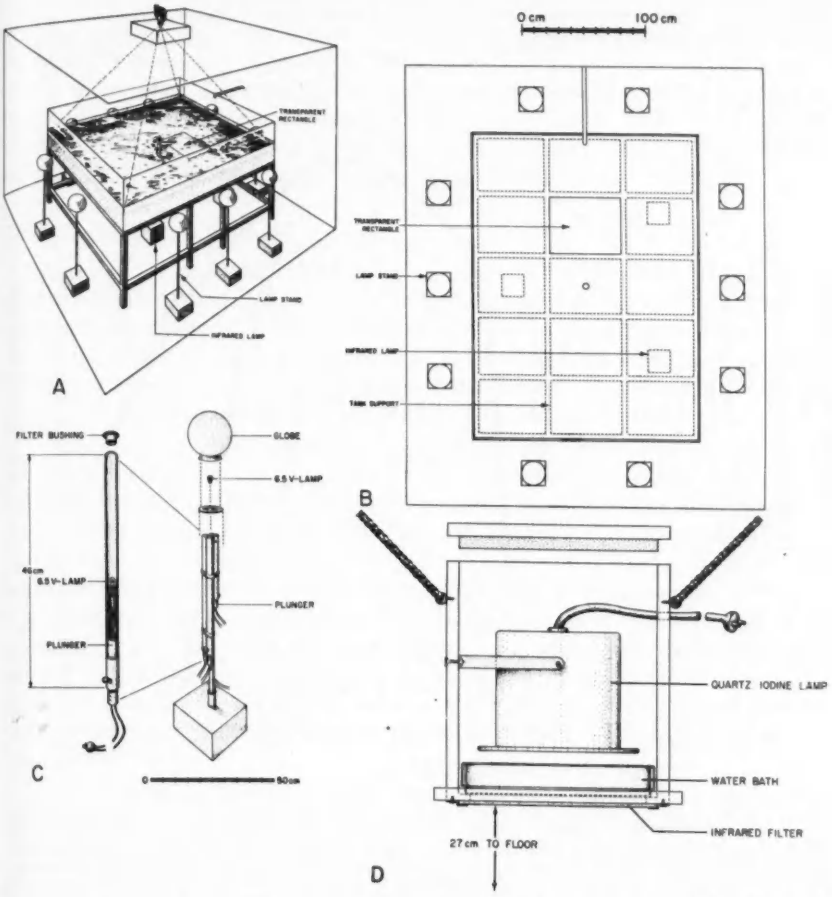

FIG. 1. Apparatus used to determine the effects of luminance on schooling and feeding behavior of jack mackerel. A, three dimensional drawing of room and tank; B, orthographic scale drawing of tank and room; C, detail of lamp stand; and D, detail of infrared lamp used for photographic illumination.

During any experiment the infrared lamps were kept on for 0.8 sec of each minute while a photograph was taken. Although the fish might have been capable of using the infrared radiation, the infrared lamps were on for such short periods that it seems doubtful that any error was introduced.

PHOTOMETRIC UNITS

The behavior of jack mackerel was studied in relation to luminance (photometric brightness) in the tank rather than to illuminance (light intensity). The luminance of a surface in foot-Lamberts is equal to the product of its

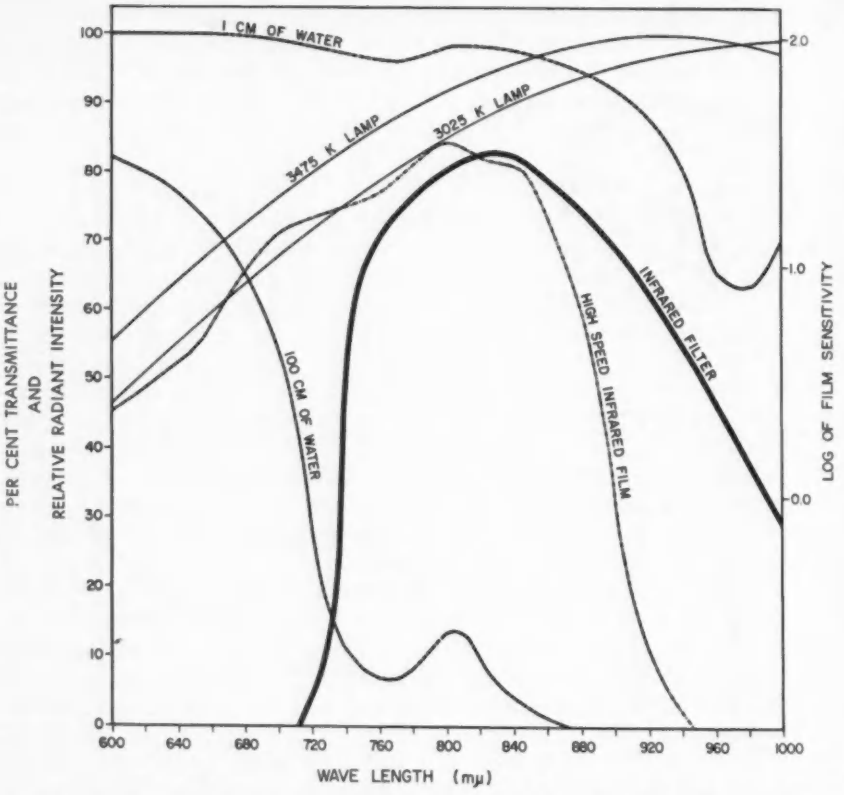

FIG. 2. Relation of the spectral sensitivity of high speed infrared film to: the spectral transmittance of the infrared transmitting filter (manufacturer's specifications); the spectral transmission of light through 1 and 100 cm of water (from Withrow and Withrow, 1956); and the relative spectral irradiance of tungsten lamps of 3025 and 3475 K (from Withrow and Withrow, 1956).

luminous directional reflectance multiplied by its illuminance in foot-candles. In all previous studies of light and schooling, measurements of illuminance were used rather than luminance. The luminance of surfaces in the path of sight is a better measure of the adaptive state of the eye than are measurements of the downwelling illuminance; for example, two containers that received equal illuminance could produce different adaptive states if the reflectances of the surfaces within the containers differed (Duntley, 1960).

It is impractical to convert the photometric measurements of intensity reported in the literature to luminance because the reflectivities of the environments are unknown. The containers varied widely: some were of concrete, others were of glass, and some had opaque white walls. Such differences in tank types might easily produce marked differences in the adaptive state of

the eye even though the illuminances entering the tanks were equal. Thus, for rough comparisons of my data with values in the literature, it seems preferable to consider the foot-candle measurements made in previous studies to be equivalent to the foot-Lambert measurements made here. It should be kept in mind, however, that in the same environment these two units would be equal only if the surfaces reflected light perfectly.

Photometric Procedures

A Gamma Scientific 700 log linear photometer provided with a 931A photomultiplier and a 180° cosine collector was used to measure levels of luminance in the tank. The photometer was calibrated in absolute units with a Gamma Scientific luminance standard, 100 ft-L at 2854 ± 50 K. A photopic filter was inserted in the optical path of the receptor to adjust the response of the 931A photomultiplier to the standard luminosity curve.

Measurements were made through the bottom of the tank at the unpainted window or within the tank 6 cm below the water surface. Measurements made through the bottom were multiplied by a constant to make them equal to those made within the tank. Each neutral density filter or combination of filters used in the calibration was calibrated on an optical bench. The luminance within the tank was measured each time a test was conducted.

Manufacturer's specifications indicated an accuracy characteristic of the photometer of ±2% when the instrument was calibrated against the luminance standard. Considering all sources of error during the calibration process, the error for most levels was ±10%. At 6×10^{-7} ft-L the error increased to ±15–20% because a higher voltage setting was used (1110 v) and the instrument was less stable.

Measurements were also made with a receptor which had a 2° acceptance angle to determine the extent of variability in luminance within the tank. The receptor was rotated on its axis under water, and readings were made at 5–10° intervals. An example of these readings is shown in Fig. 3, where the set of values at 6×10^{-1} ft-L was plotted on tracings of two underwater photographs taken with a camera which had a 180° lens. Thus, each field of view shown in the illustration resembles that seen through one eye of a fish. Photographs were also taken of a jack mackerel in the tank and the relative luminance of the fish was calculated from the optical density of the image.

Luminance within the tank did not vary greatly. The least bright area in the fish's field of view, parts of the bottom directly above the tank supports, were only 29% darker than the brightest region, the ceiling of the room as viewed by a fish through its window in the water surface as described by Walls (1942). The fish was darker than its background; the darkest areas of the body were the darkly pigmented back and the pupil and the brightest were the silvery sides and belly. The pupil had the greatest contrast with the background.

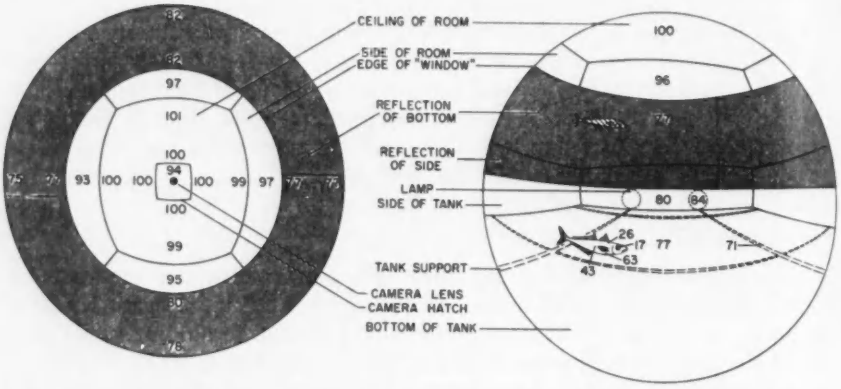

FIG. 3. Distribution of luminance viewed from within experimental tank at 6×10^{-1} ft-L. Simulations of view by a fish eye were made with a 180° "fisheye" lens. *Left panel*, hemispherical projection of visual field if path of sight is directed upward from bottom; hatched area is reflection of bottom on the silvery underside of water surface; and area within inner circle is portion of room visible through the "window" in the water surface. *Right panel*, hemispherical projection of visual field if path of sight is directed toward one end of tank from the center; circles of broken lines indicate positions of lamps outside tank wall; bands of broken lines indicate positions of supports beneath tank bottom.

LIGHT AND SCHOOLING

METHODS

Four groups of six jack mackerel each were tested at nine levels of luminance: 6×10^{-7}, 6×10^{-6}, 6×10^{-5}, 6×10^{-4}, 6×10^{-3}, 6×10^{-2}, and 6×10^{-1} ft-L, daylight (12.1 ft-L), and darkness (an unknown level of luminance below the sensitivity of the photometer). The length of the fish ranged from 105 to 141 mm standard length and the temperature in the tank varied from 17.2 to 21.0 C.

Each group was placed in the experimental tank 3 days before its first test. The daily light schedule in the tank during the acclimation period was: 0900–1000 hr, 13.5×10^{-2} ft-L; 1000–1800 hr, 12.1 ft-L; 1800–1900 hr, 13.5×10^{-2} ft-L; and 1900–0900 hr, darkness. The fish were fed frozen adult brine shrimp at 1200–1300 hr daily and the tank was cleaned at 1500–1600 hr. This schedule was continued after the tests started except that one of the test levels of luminance was substituted for 13.5×10^{-2} ft-L at 0900 hr. Only one level of luminance was tested per day, the order of the tests was random, and groups were tested only once at each level. Groups remained in the tank 12–20 days.

During each test single infrared photographs were taken at 1-min intervals from 0800 to 1100 hr. This schedule provided a photographic record of the activities of each group for 1 hr in the dark, 0800–0900 hr; for 1 hr at a randomly determined test level of luminance, 0900–1000 hr; and for 1 hr at the daytime luminance of 12.1 ft-L, 1000–1100 hr. Usually the photographs of the first 50 min of each of the three 1-hr periods were analyzed. Occasionally, some of the first 50 frames had to be discarded because of poor picture quality

and the analysis was extended to include an equal number of frames from the remaining 10 min of a 1-hr photographic sequence.

Three indices of schooling behavior were calculated from each frame: *mean separation distance*, the average of the horizontal distances separating each fish from every other fish in the school; *mean distance to the nearest neighbor*, the average of the horizontal distances from each fish in a school to its nearest neighbor; and *mean angular deviation*, the average number of degrees each fish deviated from the resultant direction of the group. The first two, mean separation distance and mean distance to the nearest neighbor, are measurements of the compactness of a school; the last, mean angular deviation, measures the angular differences in heading among individuals in a school. These indices permitted an objective evaluation of changes in schools independent of definitions of schooling behavior. The limitation, and details of calculation, of the indices have been described by Hunter (1966). Indices of schooling similar to these and which also take into account movements in the vertical plane have been developed by Dambach (1963) and Cullen et al. (1965).

Spacing and Angular Deviation of Schools

In darkness no schooling occurred. The orientation of the groups was random; no difference ($P < 0.05$) existed between the mean angular deviation of any group in darkness and the one calculated for 1 million randomly determined angles for six fish (Kolmogrov-Smirnow one sample test, Siegle, 1956). The median of all values of mean angular deviation in darkness (Fig. 4) was the same in each group as the median for the random distribution, 64°. In darkness the fish were widely distributed; the mean distance to nearest neighbor and mean separation distance in the dark were always higher than they were under brighter conditions (Fig. 5, 6). Groupings larger than pairs were rare in the photographs taken in the dark, and even pairs were so infrequent that their occurrences probably were by chance.

At 6×10^{-7} ft-L the three indices of schooling were different ($P < 0.01$) from those recorded in darkness: the mean angular deviations of groups 1, 2, and 3 differed from the random distribution; the mean separation distances of all groups differed from those of the preceding dark period; and the mean distances to nearest neighbor of groups 1, 3, and 4 differed from those of the preceding dark period. At 6×10^{-7} ft-L fish did not maintain groupings consistently and did not respond immediately to the increase in luminance. For example, in group 1 the distribution of fish during the first 15 min of the test period resembled that in darkness; no grouping larger than a single pair of fish was photographed. During the next 35 min, a grouping of three fish, then one of five, and then another of five formed and dispersed.

At 6×10^{-6} ft-L, 1 min after the lights were turned on, groupings were formed in which the distances between individuals were less than those in the dark. Within 3–4 min after the lights were turned on the fish had formed a compact, discrete school, in which the distances between fish were small and the orientations of individuals was non-random and did not vary greatly. No changes occurred during the rest of the test.

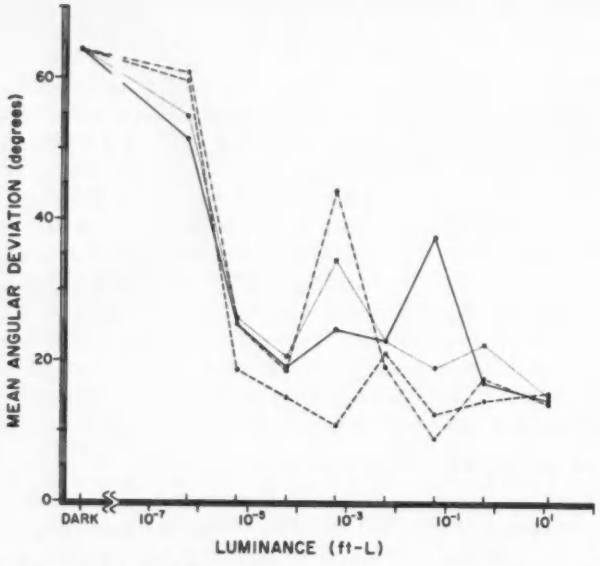

FIG. 4. Relation between luminance (logarithmic scale) and mean angular deviations of four groups of jack mackerel. Marks above abscissa indicate positions of data points; marks below indicate logarithmic scale. Points are medians of 50 values of mean regular deviation; lines connect the medians for each group.

Thus, within an increase of one order of magnitude of luminance, at the relatively low levels 6×10^{-7} to 6×10^{-6} ft-L, the schooling response of jack mackerel changed from one in which groupings of various sizes were maintained for short periods to one in which all fish remained in a compact school throughout a 1-hr period. At higher test levels of luminance (e.g. 6×10^{-1} ft-L) schools were essentially the same as those formed at 6×10^{-6}.

From 6×10^{-6} to 6×10^{-1} ft-L the distances between fish (Fig. 5, 6) were the same, but the angular deviations of the schools at 6×10^{-4} and 6×10^{-2} (Fig. 4) were variable. The reason for the increased variability of the angular deviation of schools at these two levels of luminance is unknown. It may be related to a shift from scotopic to photopic vision which may occur within this range.

In all groups, the medians for mean separation distance and mean distance to nearest neighbor were higher in the daylight sequence than in the preceding sequence ($P < 0.05$). That the distances between fish increased in daylight may have been the result of food deprivation. Hunter (1966) demonstrated that the distances between fish in jack mackerel schools increased with food deprivation.

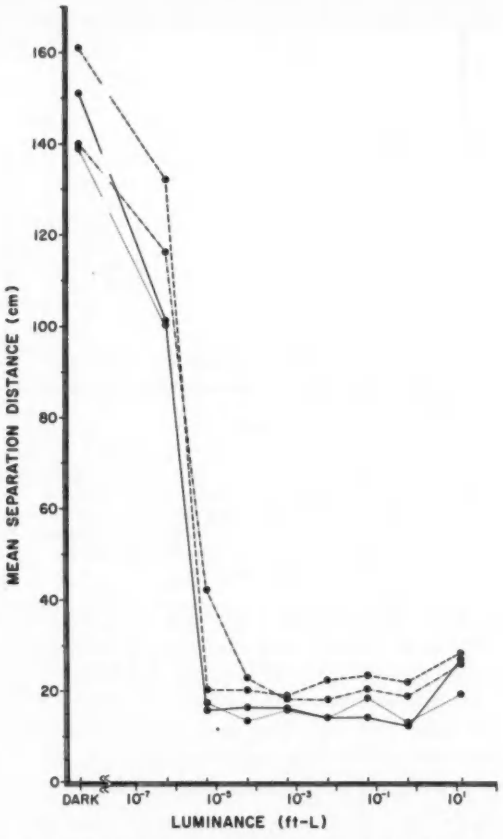

FIG. 5. Relation beween luminance (logarithmic scale) and mean separation distances of four groups of jack mackerel at various levels of luminance. Marks above abscissa indicate positions of data points; marks below indicate logarithmic scale. Points are medians of 50 values of mean separation distance; lines connect the medians for each group.

LIGHT AND FEEDING

The ability of juvenile jack mackerel, 93–143 mm standard length, to feed on live adult brine shrimp, *Artemia salina*, was tested at the nine levels of luminance used in the tests on schooling and under the same light cycle. At 0955 hr, 55 min after a test level of luminance began, live adult brine shrimp were introduced into the tank through nozzles in the center of each tank wall. All the shrimp entered the tank in 2–3 sec and were distributed uniformly at a density of 1×10^4 per m^3. At 1000 hr fish in the group were killed and shrimp in the gut of each fish counted.

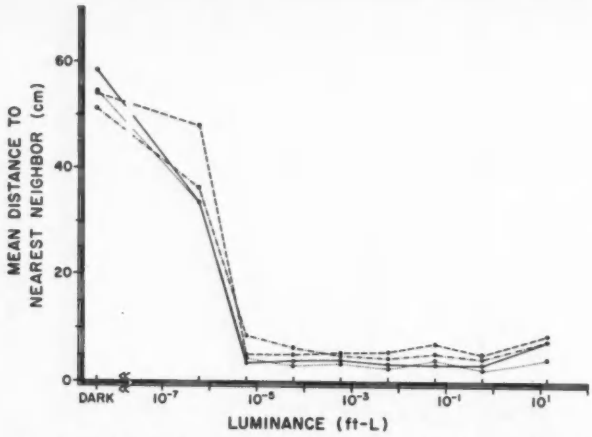

FIG. 6. Relation between luminance (logarithmic scale) and mean distances to nearest neighbor of four groups of jack mackerel at various levels of luminance. Marks above abscissa indicate positions of data points; marks below indicate logarithmic scale. Points are medians of 50 values of mean distance to nearest neighbor; lines connect the medians for each group.

Groups were placed in the tank 4 or more days before a test. They were fed daily at 0955 hr except that no food was given 48 hr before a test. One group of six fish was tested at each level of luminance except that two were tested at 6×10^{-6} ft-L because this level appeared to be near the threshold. Unlike the experiments on light and schooling, no photographs were taken and both the test in darkness and that at the daylight level (12.1 ft-L) were run at the same time of day as the rest of the tests, 0900–1000. The water temperature ranged from 19 to 22 C.

The techniques used to measure feeding behavior were adapted from those used on the northern anchovy by C. P. O'Connell (MS, in preparation) and are discussed in detail in his manuscript. His studies show that the number of adult *Artemia* consumed by an anchovy is linearly related to the length of the fish. Therefore, I adjusted the number of *Artemia* consumed for fish body length and expressed it as the number of *Artemia* consumed by a jack mackerel, 117 mm standard length, the mean length of the fish tested.

The first definite indication that insufficient light influenced the number of *Artemia* consumed was at 6×10^{-5} ft-L (Fig. 7). At one order of magnitude lower, 6×10^{-6} ft-L, the number consumed approached zero. Of the 12 fish tested at this level, 7 captured no shrimp, 4 consumed 3–9, and 1 fish captured 27. No fish fed in darkness. The extinction level probably was close to 6×10^{-7} ft-L because at this level the stomachs of three fish were empty and those of the other three contained only one or two shrimp each.

The shape of the curve for light and feeding resembled that for light and schooling but their regions of maximum slope differed. The maximum slope

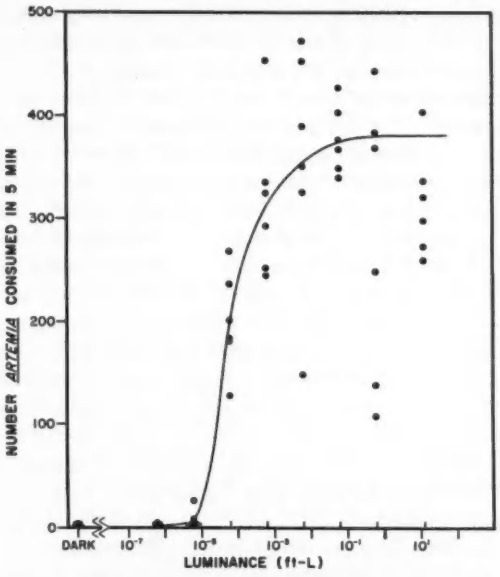

FIG. 7. Relation between luminance (logarithmic scale) and the number of adult brine shrimp, *Artemia*, consumed in 5 min by jack mackerel; each point is the number consumed by one fish after the value was adjusted for fish length. Marks above abscissa indicate positions of data points; marks below indicate logarithmic scale.

for the feeding curve was between 6×10^{-5} and 6×10^{-6} ft-L whereas that for schooling was one order of magnitude lower, between 6×10^{-7} and 6×10^{-6}. The difference in size and visibility between a brine shrimp and a jack mackerel might explain the difference in the luminance requirements for feeding and schooling.

ESTIMATES OF TRESHOLDS AND COMPARISON WITH VALUES IN THE LITERATURE

The luminance threshold for schooling was probably between 6×10^{-7} and 6×10^{-6} ft-L. If maintaining groupings for 50% of the time were used as a threshold, the schooling response at 6×10^{-7} ft-L would be considered to be below threshold because the medians for all indices in all groups at this luminance were only slightly different from the values obtained in the dark and the fish maintained groups of three or more fish for less than 50% of the time. Determination of the actual threshold value would require additional observations between 6×10^{-7} and 6×10^{-6} ft-L and the establishment of a threshold criterion for schooling.

The data on feeding were more difficult to interpret because they were more variable and because the response changed over a wider range of lumi-

nance. Any statement regarding a luminance threshold for feeding would have to be restricted to the density and size of prey used in the experiments. A criterion for threshold based on the presence or absence of brine shrimp in the gut would place the threshold near 6×10^{-7} ft-L. On the other hand, if 50% of the average number of brine shrimp captured in daylight, 12.1 ft-L, were used as a threshold it would fall between 6×10^{-5} and 6×10^{-6} ft-L. The latter criterion seems more reasonable because, if enough time were allowed, all fish held at 6×10^{-7} ft-L would probably have captured at least one brine shrimp.

The values of minimum illumination for schooling for other species summarized from the literature by Shaw (1961) and by Blaxter (1964) seem too high when they are compared with the values determined for jack mackerel. The value of 1×10^{-6} ft-c for minimum schooling by *Astyanax*, given by John (1964), is similar to the values for jack mackerel. John's data, however, showed a gradual decrease in schooling over a wide range of decreasing intensities whereas in jack mackerel the change in schooling occurred over a change of one order of magnitude. The change in jack mackerel might be expected if the luminance threshold for schooling is merely the minimum adaptive luminance required for fish to see other fish. Visual acuity in humans, for example, changes very rapidly as the light intensity is increased above threshold (Pirenne and Denton, 1952). The gradual decrease in schooling found by John (1964) suggests that light-dependent factors other than visual perception may also influence schooling in some species. The existence of factors other than visual perception may be indicated by the thresholds for schooling determined by Steven (1959) and Shaw (1961) because their threshold values appear to be too high to be near the absolute visual threshold.

The results of the feeding experiments agreed more closely with the values in the literature summarized by Blaxter (1964) than did the schooling data, probably because criteria for measuring feeding were basically the same (all were based on the number of prey captured within a certain period) whereas the criteria for measuring changes in schooling differed greatly. Most of the species listed by Blaxter (1964) seem to be able to capture significant numbers of prey at light intensities as low as 1×10^{-5} to 1×10^{-4} ft-c. The lower limit for effective feeding in jack mackerel, 6×10^{-5} ft-L, is of the same order of magnitude.

SCHOOLING AND FEEDING AT NIGHT IN THE SEA

I have chosen two points of reference to relate the behavior of jack mackerel observed in the laboratory to what might be expected in the sea: 6×10^{-6} ft-L as the adaptive luminance for schooling, and 6×10^{-5} ft-L as that for effective feeding. A number of assumptions must be made in order to relate fish behavior in the laboratory to their behavior in the sea. The experiments showed that jack mackerel can school at 6×10^{-6} ft-L but one must assume that the fish would show this behavior under natural conditions, and that their responses were not altered substantially by size and other characteristics of the laboratory environment.

Other problems and assumptions exist because of differences between photometric measurements made in the laboratory and measurements of light in the sea, and because of differences in spectral sensitivity between the human eye and the eyes of fishes. This problem has been discussed in detail by a number of authors (e.g. Seliger and McElroy, 1965; Westlake, 1965). Photometric measurements (foot-candles, foot-Lamberts) require that the photometric instrument receive and measure light over wave lengths corresponding to the average spectral sensitivity of a light-adapted human eye (the photometric standard observer). Most measurements in the sea, on the other hand, have been made in radiometric units (μ watts/cm^2), and the light entering the receptor is restricted to a narrow bandwidth. To convert radiometric measurements to photometric the spectral irradiance distribution at various depths for different water types must be known.

Spectral irradiance as a function of depth is available for Crater Lake (Smith and Tyler, 1967) and the Gulf of California (Tyler and Smith, 1967); the diffuse attenuation coefficients at a depth of 5–10 m are $K = 0.052$ m^{-1} for Crater Lake and 0.151 m^{-1} for the Gulf of California. The clarity of the water in the Gulf of California measurements is reasonably close to that found by Duntley (1959) in the vicinity of the Coronado Islands, an area inhabited by jack mackerel: $K = 0.18$ m^{-1}, at a depth of 3–10 m.

Richard Whitney (unpublished MS) prepared a series of graphs from the data of Tyler and Smith (1967) and Smith and Tyler (1967) in which the downwelling illuminance in foot-candles from 0 to 1200 m can be estimated from the solar radiation at the surface.

For a moonless starlit night Whitney's graphs show that a downwelling illuminance of 6×10^{-6} ft-c exists at a depth of 20 m in Crater Lake and at 10 m in the Gulf of California. On a moonless night the adaptive luminance in foot-Lamberts in the upper hemisphere of the visual field would probably be the same as the downwelling illuminance in foot-candles. On the basis of this evidence it appears that jack mackerel would be able to maintain schools near the surface on a moonless starlit night. Effective feeding on a moonless night would not appear to be possible because the downwelling illuminance just below the surface would be 1.5×10^{-5} ft-c.

When the full moon is at the zenith a downwelling illuminance of 6×10^{-5} ft-c would be present at 150 m in Crater Lake and at 50 m in the Gulf of California water (Whitney, unpublished MS). Under moonlight the adaptive luminance would not be equivalent to the downwelling illumination because the field would be brighter in the direction of the moon and darker in the opposite direction. Nevertheless, it seems likely that on a full moonlight night the adaptive luminance near the surface, at least in the brighter areas of the field, would be sufficient for effective feeding.

More visible energy may be available than the calculations indicate. Jack mackerel rhodopsin transmits maximally at 495 mμ (Munz, MS, 1957) and is closer to the maximum transparency of clear ocean water than is the photopic filter, which peaks at 555 mμ. Thus, in clear ocean water jack mackerel

may be able to school at greater depths than Whitney's (unpublished MS) values indicate because they are based on the photopic curve for the average human observer. In addition, bioluminescence could increase the background brightness significantly and, thereby, improve visibility.

ACKNOWLEDGMENTS

John Taylor and John Tyler of the Visibility Laboratory, University of California, San Diego, Calif., and Frank Hester, U.S. Bureau of Commercial Fisheries, La Jolla, Calif., provided valuable advice on the measurement of light. The characteristics and design of the lighting system were based on suggestions made by John Taylor. I thank John Tyler and Ray Smith for allowing me to use their unpublished spectral irradiance data and Richard Whitney for use of his photometric conversions of their data. Kenneth John, Evelyn Shaw, and Richard Whitney read the manuscript and offered helpful suggestions. Jon Van Olst gave valuable assistance in all phases of this study.

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NOTES

Distribution and Nomenclature of North American Kokanee, *Oncorhynchus nerka*

Kokanee, freshwater populations of *Oncorhynchus nerka* (Walbaum), are indigenous to lakes of the Pacific drainages in North America and northeastern Asia. In North America, kokanee occur naturally in Alaska, Yukon Territory, British Columbia, Washington, Idaho, and Oregon, and their distribution is overlapped by that of sockeye salmon, anadromous *O. nerka*.

This note assembles the available locality records for indigenous North American kokanee populations. An examination of the distribution of kokanee supports Ricker's (1940) hypothesis that most kokanee populations have been independently derived from sockeye. Conflicting ideas concerning the nomenclature of kokanee are reviewed.

Distribution.— Locality records for kokanee are given by Suckley (1861), Dawson (1879), Bendire (1878), Bean (1891), Green (1893), Jordan (1893), Evermann (1896), Evermann and Meek (1897), Babcock (1903), Chamberlain (1907), Dymond (1936), and Nelson (MS, 1960). Data from these papers, museum records (Nelson, 1968), and information from government agencies were evaluated and kokanee populations believed to be indigenous and the present distribution of sockeye are indicated in Fig. 1 and 2. In their southern range kokanee occur in most lakes where sockeye are present (Owikeno, Cultus, Chilko, Taseko, and Harrison lakes, B.C., may not contain self-sustaining kokanee), but in Bristol Bay, Cook Inlet, Prince William Sound, and Kodiak Island drainages, Alaska, where sockeye are numerous, kokanee are rare or absent.

It seems probable that sockeye once occurred in all localities where allopatric kokanee populations are definitely indigenous. Sockeye have presumably become extinct in many lakes, which now have kokanee, due to the formation of falls resulting from landform changes and the rising coastal area. For example, in the last 80 years sockeye have been barred from the Upper Columbia (i.e. Okanagan and Arrow lakes and Snake River) and some smaller river systems. Evidence exists of their extinction 150–300 years ago by natural means in three lakes which presently contain kokanee. Sockeye probably at one time migrated into Kathleen Lake, Yukon Territory (Wynne-Edwards, 1947), but were later blocked by Lowell Glacier on the Alsek River (Kindle, 1953). Sand and Lava lakes in the Nass River system, B.C., had runs of sockeye (Aiyansh Indians, personal communication) which were blocked by a lava flow. Hanson (1923) believed a lake existed at the present site of Lava Lake.

J. Fish. Res. Bd. Canada, 25(2): 409–414, 1968.
Printed in Canada.

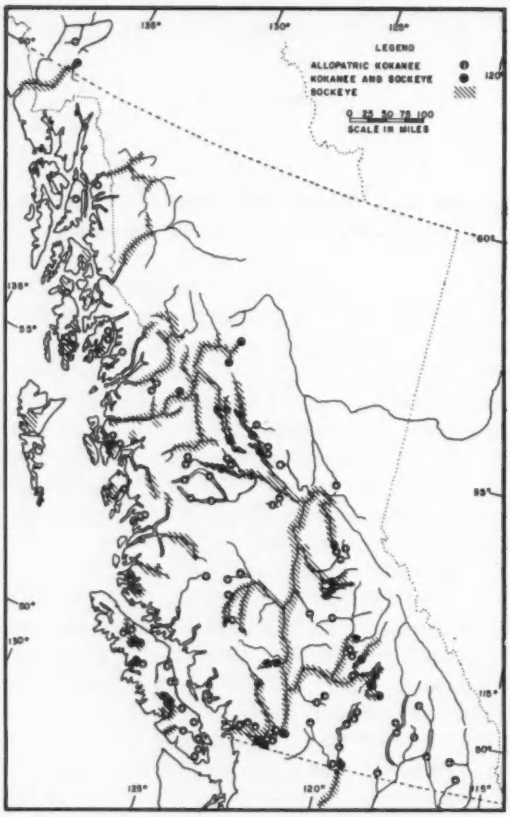

FIG. 1. Locality records of indigenous kokanee in Alaska, Yukon Territory, and British Columbia, and present distribution of sockeye in above area. Kokanee are also indigenous in Big Kitoi Lake, Alaska — 58°11′, 152°21′; and Skilak Lake, Alaska — 60°20′, 150°30′.

There is little evidence in favor of a general origin of kokanee from other kokanee populations rather than from sockeye. River piracy (natural diversion of a tributary from one river system to another) may explain the origin of some kokanee such as those in Arctic Lake (Peace River drainage) and in lakes of southeastern British Columbia. However, kokanee occur on coastal islands and in coastal mainland areas where river piracy is unlikely. Their distribution is unlike that of any other freshwater fish which lacks a diadromous form. Large *O. nerka* attempt to surmount impassable falls below several lakes with kokanee. Even if these individuals were the offspring of kokanee it seems improbable that they could have originated most other kokanee populations.

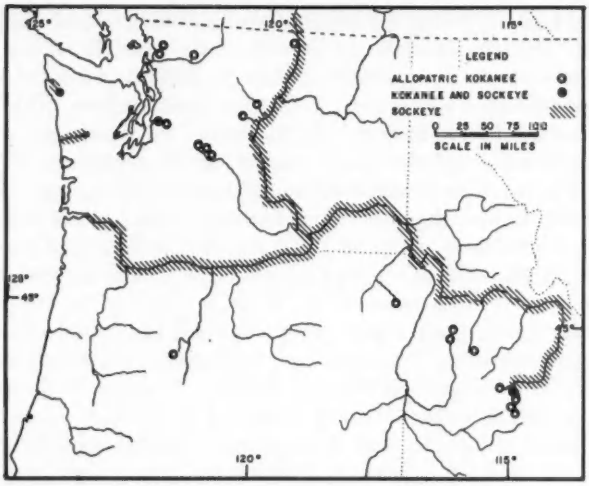

FIG. 2. Locality records of indigenous kokanee in Washington, Idaho, and Oregon, and present distribution of sockeye in above area.

Ricker's (1940) hypothesis that most kokanee populations have been independently derived from various sockeye populations is supported by the following: (1) kokanee are indigenous only in areas which have probably been frequented by sockeye; (2) the total distribution of kokanee is most plausibly explained by the presence of an anadromous form; and (3) offspring of kokanee and sockeye may, contrary to their usual life-history, become anadromous or remain in freshwater, respectively (Fraser, 1918; Ward, 1929, 1932; Foerster, 1947; Rounsefell, 1958; Ricker, 1959).

Nomenclature.— Disagreement has arisen on the nomenclatorial status of kokanee and sockeye. The various names applied to North American kokanee are presented to assist in tracing their history.

Oncorhynchus nerka (Walbaum) — Kokanee
Salmo kennerlyi Suckley, 1861
Hypsifario kennerlyi Gill, 1862
Oncorhynchus kennerlyi Jordan, 1878a
Oncorhynchus kennerlii Jordan, 1878b
Oncorhynchus nerka Bendire, 1882; Jordan and Gilbert, 1883
Oncorhynchus nerka kennerlyi Bean, 1891

Kokanee were first described by Suckley (1861) as *Salmo kennerlyi*. Gill's (1862) genus *Hypsifario* was intended to distinguish kokanee from *Salmo* by the compressed body and projected snout of the former. The name lacks priority at the generic level but was used at the subgeneric level by Jordan (1878a) for kokanee (with sockeye in the subgenus *Oncorhynchus*)

and by Jordan et al. (1930) for kokanee and sockeye. Girard (1856) accepted Valenciennes' erection of three genera for Artedi and Linnaeus' *Salmo*. He described *Fario aurora* (= sockeye salmon — Jordan and Evermann, 1896), but the generic name *Fario* is unavailable for Pacific salmon; the type species is now placed in *Salmo*. Suckley (1874) considered *S. kennerlyi* to be non-anadromous. Bendire (1882) and D. S. Jordan in Bendire (1882), however, believed that kokanee were anadromous and represented young breeding males of sockeye, and applied the name *Oncorhynchus nerka*. *Oncorhynchus* was used originally as a subgeneric name by Suckley (1861) and later as a generic name by Günther (1866). Bean (1891) concluded that the specimens on which Jordan based his decision were chiefly sockeye collected by Bendire. Indeed, when Bendire (1882) gives arguments for individuals being anadromous and not dying after spawning he mentions 5- and 10-lb fish, very likely sockeye. On the basis of presumed differences between kokanee and sockeye, Bean applied the trinomial name *O. nerka kennerlyi* to kokanee. Evermann (1896) examined a series of kokanee and sockeye from Idaho, rejected the differences Bean found, and referred to both as *O. nerka*. He did not feel there was enough evidence to state whether they were anadromous or freshwater. Jordan and Evermann (1896) also applied the name *O. nerka* to both kokanee and sockeye but for different reasons than Evermann (1896). They agreed that there was no structural difference between the two forms but incorrectly felt (presumably Jordan only — see Jordan in Bendire, 1882) that kokanee were just young individuals of sockeye which had returned from the ocean. Jordan (1893) curiously refers to *O. kennerlyi* as landlocked despite later rejecting and having earlier rejected this belief. Subsequent investigators referred to kokanee as: (*a*) *O. kennerlyi* (e.g. Halkett, 1913; Jordan et al., 1930; Ward, 1932); (*b*) *O. nerka kennerlyi* (e.g. Chamberlain, 1907; Dymond, 1936; Ricker, 1940; Foerster, 1947); or (*c*) *O. nerka* (e.g. Schultz et al., 1935; Lindsey, 1956; Bailey, 1960; Vladykov, 1962). Workers studying kokanee in Asia have divided kokanee into different species (Jordan and McGregor, 1925) or different subspecies (Berg, 1948), or have recognized only the binomial name *O. nerka* despite finding differences in certain characters between the two forms (Hikita, 1962).

The sympatric occurrence of two forms, kokanee and sockeye, each having a distinctive life-history and each able to maintain itself in allopatry, would perhaps suggest that they should be regarded as different species. Or, if full species status does not seem warranted, the desirability of taxonomically recognizing the two forms, thereby avoiding the necessity of using a vernacular name for each form and of using two vernacular names for the same taxonomic unit, would leave open the alternative of lumping all kokanee populations into one subspecies. However, the probable phenotypic basis of the partial isolating mechanisms between kokanee and sockeye, the ability of one form to produce offspring which take up the life-history of the other, and the presumed multiple origins of kokanee from sockeye make it advisable to designate both forms as *Oncorhynchus nerka*.

This study was initiated at the suggestion of Dr C. C. Lindsey. I am indebted to Dr J. C. Briggs of the University of South Florida, Miss J. Goodman of the International Pacific Salmon Fisheries Commission, Drs T. G. Northcote, N. J. Wilimovsky, and W. E. Ricker of the Fisheries Research Board of Canada, and F. J. Ward of the University of Manitoba, my wife, Claudine, and my father, Mr W. I. Nelson, for information or services. Much of the information on the distributions of kokanee and sockeye is from the International Pacific Salmon Fisheries Commission, the British Columbia Department of Fish and Game, the Department of Fisheries of Canada, the United States Department of the Interior, and the Washington Department of Game.

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Received for publication October 11, 1967.

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Variation in Gillraker Number in North American Kokanee, *Oncorhynchus nerka*

Kokanee, freshwater populations of *Oncorhynchus nerka* (Walbaum), have probably been independently derived from sockeye salmon, anadromous *O. nerka*, in most localities throughout their range (Ricker, 1940; Nelson, 1968). Self-sustaining kokanee populations may exist in sympatry with their progenitor the sockeye or in allopatry.

Although Vernon (1957) found significant differences in numbers of vertebrae, scale rows, and gillrakers between kokanee populations within Kootenay Lake, no detailed study has been made of variation between kokanee occupying different lakes. This study is concerned with gillraker variation among populations of North American kokanee.

Materials and methods.— Gillrakers were counted on kokanee from 19 localities and sockeye from 3 localities (Table I). The sockeye were collected in 1964 and the kokanee in different years. The first left gill arch was removed and the gillrakers counted under a binocular microscope. All projections lying in a near lineal series were counted. Counts were made in the same manner on the gillrakers of the second, third, and fourth arches in seven kokanee and three sockeye populations.

A previous study (Nelson, MS, 1960) and the present data showed no obvious association of the number of anterior gillrakers on the first arch with size of the specimen within a population, with sex over the size range examined, or with latitude, longitude, or altitude of the population.

Two series of possible gillraker counts were not studied in detail. Of these, in 10 kokanee and 10 sockeye examined, there were 13–15 posterior fourth arch gillrakers and 12–14 anterior fifth arch gillrakers.

Variation between year-classes.— Differences in gillraker number in seven year-classes of kokanee from Nicola Lake were slight (Fig. 1). Lorz and Northcote (1965) indicate that gene flow may be considerable between the various year-classes of this population because of variability in time of maturity. A marked increase in the average size of spawning individuals and a decrease in the average age of spawning individuals occurred during the 7 years of investigation (Lorz and Northcote, 1965), but the gillraker number remained relatively unchanged.

A comparison of unpublished data of the International Pacific Salmon Fisheries Commission with the present work also indicates time stability in the number of gillrakers. Collections of kokanee from Narrows Creek, Takla Lake (raker mean 38.2; range 36–43) in 1940 agree closely with the present data from fish collected in 1962.

Variation between populations.— Considerable variation was found in anterior gillraker number on the first four arches and the posterior gillraker

J. Fish. Res. Bd. Canada, 25(2): 415–420, 1968.
Printed in Canada.

TABLE I. Localities from which kokanee and sockeye were examined. All kokanee are in the Institute of Fisheries, The University of British Columbia, Vancouver, B.C. The sockeye were discarded. K: kokanee; S: sockeye.

Locality	N lat, W long	Form	Museum No.	No. of specimens examined	Sockeye present	Fork length(*cm*)
Alsek River System						
Kathleen Lake						
Inlet	60°33′, 137°32′	K	60-660	4	–	36–39
Nass River System						
Sand Lake	54°58′, 128°58′	K	56-620	6	–	15–25
Skeena River System						
Babine Lake						
Pierre Creek	53°37′, 125°52′	K	64-201	40	+	22–25
Link River System						
Link Lake	52°25′, 127°40′	K	59-522	6	–	17
Vancouver Island Drainage						
Hustan Lake	50°15′, 126°56′	K	57-399	4	–	12–13
Bonanza Lake						
Bonanza Creek	50°22′, 126°46′	K	59-492	3	–	14–18
Quinsam Lake	49°58′, 125°19′	K	59-118	16	–	11–17
Fraser River System						
Takla Lake						
Narrows Creek	55°09′, 125°43′	K	64-17	30	+	18–20
Gluske Creek	55°03′, 125°47′	S		30		53–65
Trembleur Lake						
Kazchek Creek	54°50′, 125°10′	K	64-18	20	+	20–22
Stuart Lake						
Tachie River	54°41′, 124°47′	K	64-19	30	+	19–21
Tachie River	54°41′, 124°47′	S		10		51–64
Pinchi Lake	54°36′, 124°30′	K	59-503	5	–	17–21
Francois Lake						
Near outlet	54°00′, 125°02′	K	64-23	20	+	22–29
Cluculz Lake	53°52′, 123°38′	K	64-198	12	–	15–24
Horsefly Lake						
Little Horsefly Creek, Upper	52°22′, 121°15′	K	64-21	30	+	24–30
Shuswap Lake						
Signal Bay	50°51′, 119°00′	K	60-23	20	+	21–26
Anderson Lake						
Near outlet	50°42′, 122°18′	K	64-25	20	+	25–29
Gates Creek	50°35′, 122°27′	S		24		55–68
Nicola Lake						
Moore Creek	50°13′, 120°27′	K			–	
1957			59-514	5		23–26
1958			58-575; 60-148, 149	21		22–26
1959			60-24	30		21–23
1960			61-90	8		26–28
1961			61-585	11		29–34
1962			64-199	30		26–30
1963			64-200	30		25–30
Columbia River System						
Moyie Lake	49°16′, 115°50′	K	56-575; 59-198	30	–	19–26
Christina Lake	49°06′, 118°15′	K	54-138	15	–	18–20

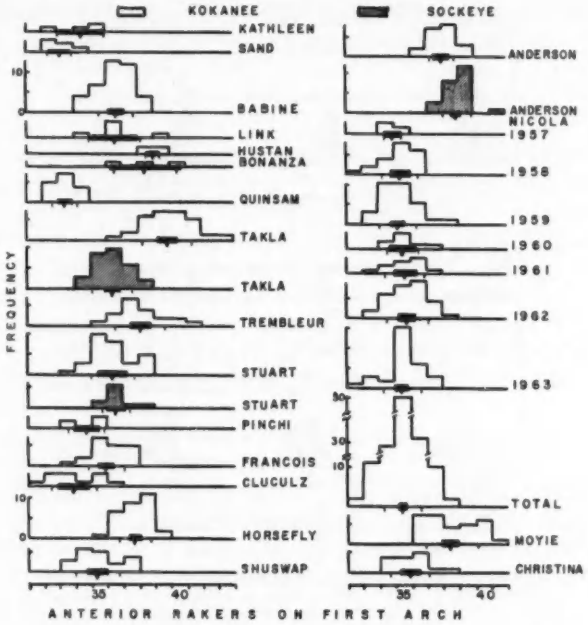

FIG. 1. Frequency distributions of anterior gillrakers on first arch in kokanee and sockeye. The mean (small triangle), two standard errors of the mean on either side of the mean (blackened bar), and one standard deviation on either side of the mean (small line) are included. Where there is no overlap in the mean ± 2 standard errors of the mean, considerable reliance can be placed on the significance of the difference between samples (see Hubbs and Hubbs (1953) for fuller discussion).

number on the first three arches between kokanee from different lakes (Fig. 1–3). Many significant differences exist. Except for the posterior first arch gillrakers there was a marked difference between the Takla and Francois populations and, in most counts, a decrease in gillraker number from Takla through Trembleur to Stuart. Other populations in relatively close proximity to each other also showed significant differences. For example, Christina differed from Moyie, Hustan and Bonanza differed from Quinsam, Horsefly differed from Shuswap, and Pinchi and Sand differed from Babine kokanee in anterior first arch gillrakers.

In the limited data several differences in gillraker number between sympatric kokanee and sockeye were found (Fig. 1–3). In all counts, except those for the posterior first arch gillrakers (Fig. 3), Takla Lake kokanee from Narrows Creek had a higher gillraker number than did sockeye from Gluske Creek, another tributary. Both kokanee and sockeye spawn in Narrows Creek, but sockeye from this area of strict sympatry were not obtained for examination. Differences at Stuart Lake between kokanee and sockeye from the same river

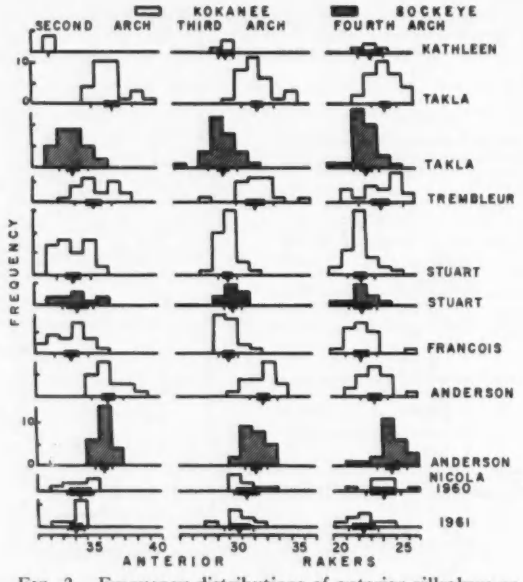

FIG. 2. Frequency distributions of anterior gillrakers on second, third, and fourth arches in kokanee and sockeye. See Fig. 1 for further explanation.

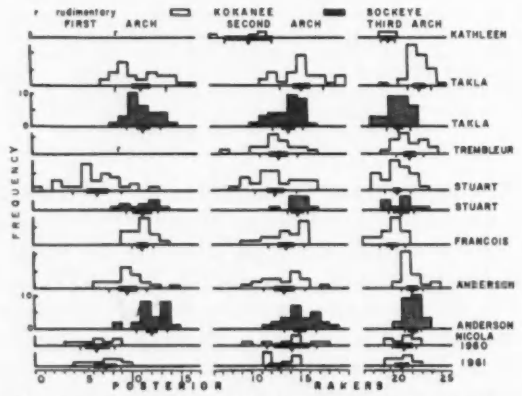

FIG. 3. Frequency distributions of posterior gillrakers on first, second, and third arches in kokanee and sockeye. See Fig. 1 for further explanation.

system were few. Only the posterior gillrakers of the first and second arches showed significant differences. In Anderson Lake there were few differences between the two forms from different areas. The kokanee had significantly fewer anterior first, anterior fourth, posterior first, and posterior second arch gillrakers than the sockeye.

The difference in means of 3.6 in the anterior first arch gillrakers between the kokanee and sockeye from Takla Lake was more than twice the greatest difference of 1.65 that Vernon (1957) found between three races of Kootenay Lake kokanee. The significant difference within one lake was postulated by Vernon to have resulted from segregation during reproduction. The history of the difference in Takla Lake may only be speculated upon. It does not appear, however, that the gillraker differences necessarily arose between forms having always occurred in sympatry. The kokanee and sockeye compared in Takla Lake were from creeks entering the lake on opposite sides. Also, most of the sockeye from Gluske Creek may be descendants of Trembleur Lake stocks. Original Takla Lake sockeye, part of the early Stuart Lake run, may have been largely obliterated by the 1913–1914 Hells Gate obstruction on the Fraser River and the remainder Takla sockeye diluted with strays from the Trembleur Lake area during exceptionally large runs in 1949 (Jan Goodman, personal communication).

The range of means of first arch gillrakers in 8 allopatric kokanee populations and 11 sympatric with sockeye (Fig. 1) encompasses the range of means of the sockeye populations given by Fukuhara et al. (1962), Rounsefell (1962), and the International Pacific Salmon Fisheries Commission (unpublished information). There was no evidence from the limited data that allopatric kokanee differ more from sockeye than those kokanee which are still sympatric and thereby perhaps having gene flow with sockeye.

McCart and Anderson (1967) have shown plasticity in kokanee gillraker number by demonstrating that kokanee reared in an artificial environment had a significantly lower number of gillrakers than their parents. However, numerous studies have shown both a genetic and a phenotypic basis for meristic characters (Barlow, 1961). The degree to which differences in gillraker number between kokanee populations may reflect genetic divergence is not known. Populations with a gillraker difference have not been reared in the same environment to determine if the difference is maintained.

This study was initiated at the suggestion of Dr C. C. Lindsey. His guidance is sincerely appreciated. Dr W. E. Ricker and Mr P. J. McCart of the Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C., and Miss J. Goodman of the International Pacific Salmon Fisheries Commission, New Westminister, B.C., gave valuable advice. The author is indebted to Drs T. G. Northcote and F. J. Ward, and to his wife, Claudine, for information or services. Most of the material is from collections made by the International Pacific Salmon Fisheries Commission.

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Occurrence of *Parasudis truculentus* (Goode and Bean) 1895 (Iniomi: Chlorophthalmidae) off LaHave Bank, Nova Scotia

A specimen of the chlorophthalmid *Parasudis truculentus* (Goode and Bean) 1895, the longnose greeneye (Fig. 1), was taken southeast of LaHave Bank (42°52′N, 63°45′W) on July 5, 1967 in 84–92 fath of water and at a bottom temperature of 7.3 C. The gear used was an otter trawl with both $1\frac{1}{4}$- and $\frac{1}{4}$-inch mesh liners in the codend.

TABLE I. Body proportions and meristic characters of Nova Scotian specimen of *P. truculentus*. Last dorsal and anal finrays bifid.

Character	Measurement	
	mm	% of standard length
Total length	118.0	
Fork length	109.0	
Standard length	99.0	
Depth at origin of dorsal fin	14.0	14.1
Depth at origin of anal fin	9.0	9.1
Width at base of pectoral fins	10.5	10.6
Least depth of caudal peduncle	7.5	7.6
Head length	32.5	32.8
Head width between midposterior points of orbit	11.5	11.6
Snout length from tip of upper jaw to nearest point of eye	13.0	13.1
Snout length from tip of upper jaw to end of maxillary	11.5	11.6
Horizontal diameter of orbit	10.5	10.6
Vertical diameter of orbit	8.5	8.6
Horizontal diameter of eye	10.0	10.1
Width of bony interorbital	4.5	4.5
Caudal fin length	19.0	19.2
Ventral fin length	19.0	19.2
Pectoral fin length	19.0	19.2
Snout to origin of dorsal fin	40.5	40.9
Snout to origin of anal fin	76.5	77.3
Snout to origin of ventral fin	41.5	41.9
Snout to anus	56.5	57.1
Fin-rays: Dorsal	10 (1)	
Anal	9 (1)	
Caudal	1–17–1	
Ventral	9; 9	
Pectoral	14; 14	
Vertebrae	38 + urostyle	

J. FISH. RES. BD. CANADA, 25(2): 421–422, 1968.
Printed in Canada.

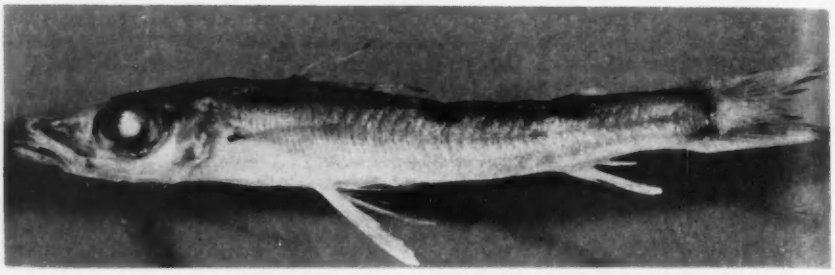

FIG. 1. *Parasudis truculentus* captured southeast of LaHave Bank, Nova Scotia.

In a review of the systematic position and distribution of this species, Mead (1966) reports that its range extends from the equator off the Amazon delta, to Nantucket, Massachusetts, where fewer than 10 specimens were recorded by Schroeder (1955). It is a common offshore resident along the southeastern Atlantic coast of the United States and in the Gulf of Mexico where hundreds of individuals are frequently caught in shrimp nets. All previously reported specimens were obtained by bottom trawling in depths of 100–260 fath, usually over a mud bottom, at temperatures of 8.9–13.3 C. The present record extends the geographical limits of the species about 300 miles to the northeast and increases both the depth and temperature ranges.

Body proportions and meristic characters (Table I) differ little from those of 20 specimens (probably almost all larger than the present specimen) captured in the Gulf of Mexico and off northern South America (Mead, 1966).

I wish to thank Dr W. B. Scott for having X-ray photographs of the fish taken and for his help in identification. The specimen is stored in the Royal Ontario Museum (Cat. No. ROM 25,552). The author is supported by a post-doctoral fellowship from the National Research Council.

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Received for publication October 16, 1967.

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Infection of Brook Trout (*Salvelinus fontinalis*) by Nocardiae

The causative organism of a tuberculosis-like disease of neon tetras (*Hyphessobrycon innesi*) (Conroy, 1963) was identified later as *Nocardia asteroides* (Valdez and Conroy, 1963). This initial record of fish nocardiosis was followed by Snieszko's et al. (1964) report of a nocardial infection of hatchery-reared rainbow trout (*Salmo gairdneri*). These infections probably occur more frequently than the few reports suggest since many cases may have been unrecognized because of fragmentation of the organism's mycelia to bacillary and coccoid forms (Heuschmann-Brunner, 1965). This note emphasizes the need for an awareness of the role of nocardiae in fish pathology and records the first nocardial infection of brook trout (*Salvelinus fontinalis*).

Observations.— One 9-inch brook trout died in our freshwater aquarium in mid-December 1966. Autopsy revealed that the kidney was brown with a granular texture, particularly in the posterior portion. Subsequent difficulties with the aquarium water supply resulted in the death of the remaining trout held in this tank. Considerable degeneration of the kidney was observed in every fish. The posterior third of the kidney in the most extreme case had become hard and rubbery and in three fish the gonads had hemorrhaged.

Isolation and identification.— Kidney samples from each fish were swabbed on Trypticase soy agar (TSA) plates which were then incubated at 18 C. The growth at 21 days consisted of white to faintly pink colonies (up to 2 mm diam) with considerable variation of the ridges and folds of the colonies' surfaces. Subculture of 40 representative isolates on TSA produced convex colonies (up to 1 mm diam) all of which elaborated an amber-coloured water-soluble pigment. The 40 isolates were gram positive, non-acid-fast, obligate aerobes, possessing true mycelia with aerial hyphae. Fragmentation of mycelia was readily apparent in older cultures. Partial characterization of all the isolates was followed by a detailed study of one representative isolate (N 49).

The methods of Gordon and Mihm (1962) were employed for characterization of N 49 except that lysozyme was not pretreated with acid. Results of these tests were recorded after a maximum incubation of 28 days at 28 C. Paraffin utilization was determined using the medium of Bushnell and Haas (1941). Procedures described in the Manual of Microbiological Methods (Society of American Bacteriologists, 1957) were used in additional tests (Table I). In contrast to the 142 strains of *N. asteroides* studied by Gordon and Mihm (1962), our cultures have grown at a range of 10–28 C but not at all at 35 C. Except for the inability to utilize pyruvate as the sole carbon source, the properties of N 49 conformed to the 25 physiological characteristics proposed for *N. asteroides* (Gordon and Mihm, 1962). Therefore, we conclude N 49 is *N. asteroides*.

J. Fish. Res. Bd. Canada, 25(2): 423–425, 1968.
Printed in Canada.

TABLE I. Additional physiological characteristics of N 49.

Test	Results
Utilization of paraffin	+
Hydrolysis of starch	+
Nitrite from nitrate	+
Hydrogen sulfide production	−
Lipolysis	−
Litmus milk	Alkaline becoming reduced
Gelatin stab	No liquifaction, surface growth only
Growth (*C*) at 35	−
28	+
25	+
18	+
10	+
4	−

In vivo experiments.— Intraperitoneal injection of 0.5-ml saline suspension into yearling brook trout was carried out at two dosage levels. Sterile saline (0.5 ml) was used to inoculate controls. During the 100-day duration of these experiments the water temperature ranged from 6 to 10 C.

The three trout, each of which received an inoculation of approximately 4 mg dry-weight of cells of N 49, survived the 100-day test period, as did the controls. Forty days after inoculation a lesion surrounding the injection site (Fig. 1) was evident in one fish; it had completely regressed by the 100th day. Nocardia were found in the tissues and peritoneal fluid from the three inoculated fish.

Concurrently three additional fish were injected with an inoculum 20 times more concentrated than that recorded above. One fish died 43 days after inoculation and a second fish died 13 days later. Both fish were swollen as a result of a large amount of body fluid. Numerous distinct colonies of nocardiae were found on the kidney, liver, gonads, ventral body wall, and in both the heart cavity and the brain. Necrotic damage to the visceral organs was not evident although we observed extensive hemorrhage and degeneration of the ventral body wall (Fig. 1). No signs of disease were detected in the third fish which survived the 100-day test period. However, a typical lesion surrounding the injection site had been evident during the course of the experiment. Nocardiae were recovered from the internal organs of all the infected trout. Control fish remained healthy.

Discussion.— These results are consistent with the hypothesis of Heuschmann-Brunner (1965) that nocardiae are pathogenic for fish only under certain conditions. The role of stress factors in converting low-grade infections into acute disease forms is well recognized and further work should include an examination of these factors for a better understanding of the role of nocardiae in fish pathology.

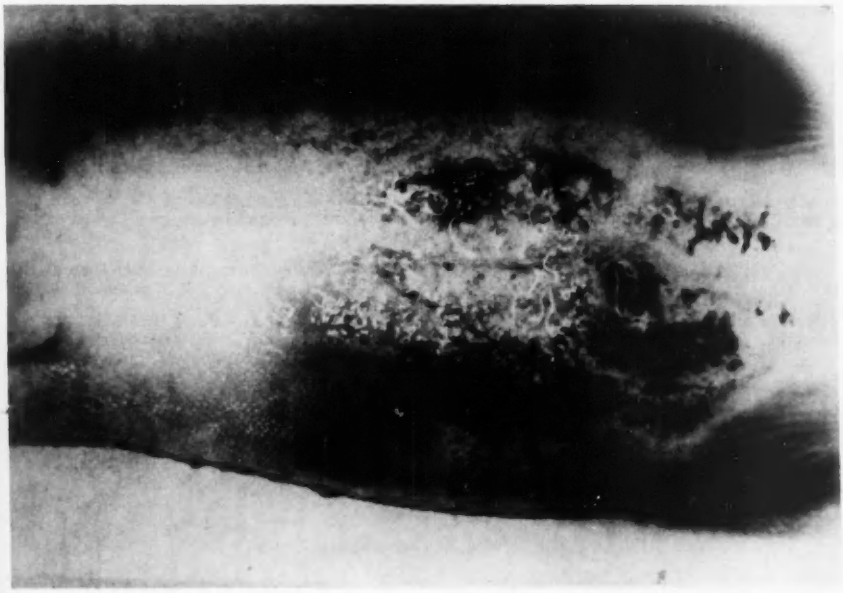

FIG. 1. View of the ventral surface of a brook trout showing a typical lesion caused by nocardial infection.

The authors thank Dr James E. Stewart for his critical review of the manuscript and Mr H. E. Power for help in the preparation of the photograph.

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Received for publication September 8, 1967.

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Distribution of Lobster Larvae (*Homarus americanus*) off Pictou, Nova Scotia

The announcement that a bleached-kraft pulp mill was to be constructed at Abercrombie Pt. near Pictou, N.S. (45°41′N lat, 62°42′W long) caused concern to lobster fishermen of the area. Plans called for Boat Harbour (Fig. 1) to be converted into a waste treatment lagoon receiving effluent from the mill and discharging periodically into the sea. It was thought that lobster larvae (*Homarus americanus*) might encounter lethal concentrations of the freshwater effluent, which in time would affect commercial lobster catches in the area.

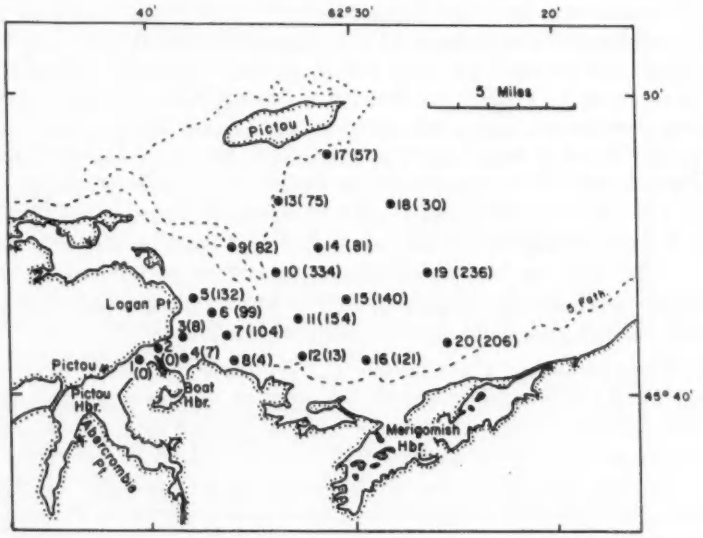

FIG. 1. Survey area showing location of stations and the number of stage I lobster larvae captured at each.

Templeman (1937) and Smith (MS, 1937) showed that larvae normally concentrated at the water surface and were transported by wind-driven surface currents.

This paper describes the distribution of lobster larvae in the sea off Pictou and explores the extent to which lobster larvae may encounter the effluent.

The survey area consists of a wide bay opening towards the northeast and partially protected by shallow areas extending out to Pictou Island. Pictou Harbour empties into the area from the southwest and the entrance

J. FISH. RES. BD. CANADA, 25(2): 427–430, 1968.
Printed in Canada.

to Boat Harbour is about 1 mile east of this. Twenty stations were each occupied five times during the period July 25–August 3, 1966. All tows were made during daylight.

The net (Wilder, 1953; Scarratt, 1964) measured 12 × 3 ft (3.7 × 0.9 m) at the mouth by 25 ft (7.5 m) long and was towed breaking the surface. The net material was nylon "Nitex" approximately 18 mesh/inch (7 mesh/cm). Each tow was of ½ hr duration and approximately ½ nautical mile was covered. The area fished by one tow was therefore of the order of 4000 yards^2 (3340 m^2) or about 1/1000 nautical mile^2. At the beginning of each tow, notes were made of wind speed and direction, cloud cover, weather, and surface water temperature. Surface water salinity was obtained from hydrometer readings taken during the first 31 tows.

Surface hydrographic conditions.— Surface salinity in the study area during the first 3 days of the survey period ranged from 27.7‰ to 28.9‰. The less saline water was found off Pictou Harbour and off Merigomish Harbour with higher salinity water between and to seaward of these patches. Rainfall for the month of July had been less than average for the region and there were no heavy showers during the study period (Canada Department of Transport, 1966). It is not likely that the salinity changed appreciably during the study period. Winds recorded at sea were predominantly southwesterly, which is typical for that time of year (Canada Department of Mines and Technical Surveys, 1957). There were no strong onshore winds during the survey period and the larval distribution should be considered as normal.

Surface temperature ranged from 15.0 to 19.7 C, the warmest water being found in Pictou Harbour and off the mouth of Boat Harbour.

Water circulation.— Farquharson (1959) has shown that the residual drift at 7 m in the general area is of the order of 1.2 miles per day towards the south. Lauzier (1965) computed a residual surface drift of the order of 1.5–3.5 miles per day from returned drift bottles released in the Pictou area, with an inferred direction towards the southeast and east. L. M. Lauzier (personal communication) considers that water passing between Pictou Island and the mainland will turn toward the east rather than enter Pictou Harbour.

Dr R. Trites (Fisheries Research Board of Canada, Dartmouth, N.S., personal communication) has found that dye released a little to the south of Logan Point moved up to and a little to the westward of the Point on the flooding tide, and then moved slowly to the southeast on the ebb. Dye released inshore at the mouth of Boat Harbour at high tide tended to split into two parts, part moving toward Pictou Harbour and the other part moving to the southeast but remaining close to shore.

Abundance and distribution of the larvae.— A total of 1984 larvae were captured in 100 tows. These consisted of: 1883 in stage I (Fig. 1), 88 in stage II, 7 in stage III, and 6 in stage IV. At 16.7 C the larvae are in stage I for 4½ days, stage II for 5½ days, stage III for 8 days, and stage IV for 19 days (Templeman, 1936). Of the inshore stations, 1 and 2 at the mouth of Pictou

Harbour yielded no larvae at all whereas stations 3, 4, 8, and 12 yielded only a few. Higher catches were made at adjacent stations, no. 5, 6, 7, and 11, and the highest catches of all were made at station 10 near the centre of the area. There thus appears to be a zone of low larval density close inshore and a zone of higher larval density further offshore. The boundary between the zones roughly follows the 5-fath contour.

Discussion.— The absence of larvae from the stations close inshore suggests that few larvae are hatched near the mouths of Pictou Harbour and Boat Harbour. It also supports Lauzier's contention that water moving south into the area is deflected to the east. Possibly many of the larvae found in the central part of the area have drifted in through the channel between Pictou Island and the mainland.

Under normal conditions the effluents discharged from Boat Harbour will be moved along the coast toward the east, but inshore of the main larval area. Scarratt and Raine (1967) have shown that newly hatched lobster larvae do not readily swim up into low salinity surface layers. Therefore, larvae hatched inshore may well remain below any patches of low salinity, effluent-bearing surface water. The pattern may be changed by strong easterly winds, which will tend to set larvae in toward the shore. Under these conditions there will be much turbulence and mixing of the shallow waters and a consequent dilution of the effluent. It is not expected that many larvae will encounter undiluted effluent, either in normal or in adverse conditions.

I am indebted to Capt P. T. Ossinger and the crew of M.B. *Pandalus II* who collected the samples, and to Mr Robert Lowe who sorted, identified, and counted the larvae.

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Received for publication August 23, 1967.

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Biosynthesis of Aldosterone and 18-Hydroxycorticosterone from Corticosterone by Interrenal Tissue of a Teleost (*Clupea harengus harengus*)

In two recent publications (Mayer et al., 1967; Bern, 1967) it has been suggested that aldosterone does not appear to be a hormone of marine vertebrates but is rather a development of the terrestrial vertebrate. The question of the presence or absence of aldosterone as a product of the interrenal tissue of teleosts has been debated for several years. In 1959, Phillips et al. isolated a compound from plasma of sockeye salmon which possessed a Δ^4-3-ketone group, reduced blue tetrazolium, and behaved chromatographically as aldosterone. However, no derivatives of this compound were prepared and its identification was not rigidly established. A steroid of similar polarity isolated from blood of the same population by one of us could not be identified as aldosterone by derivative formation (Bern et al., 1962) and it showed no biological activity for the rat (Idler et al., 1959). In vitro studies of interrenal secretion of teleosts also have provided contradictory results. Incubation of tissue from the killifish, *Fundulus heteroclitus*, with [16-^{3}H] progesterone produced a tritiated derivative which was tentatively identified as aldosterone (Phillips and Mulrow, 1959). Again this compound behaved chromatographically as aldosterone but upon acetylation its radioactivity was reduced considerably and the authors suggested further purification of the unknown was required to establish its identity as aldosterone. Recently, Sandor et al. (1966, 1967) reported the results of an extensive study of steroidogenesis in vitro of adrenocortical tissue of the eel (*Anguilla anguilla*). Although the products of the incubated tissue were examined specifically for aldosterone, there was no evidence of the transformation of corticosterone, progesterone, pregnenolone, or cholesterol to 18-oxygenated derivatives. These careful and well-controlled experiments coupled with the demonstration (Mayer et al., 1967) that cortisol has mineralocorticoid activity in the eel have cast doubt on the existence of aldosterone in teleosts. Thus, at this time we would like to report some unexpected results of an experiment originally planned to determine the distribution of the corticosteroid 1α-hydroxylating system found in elasmobranchs (Idler and Truscott, 1967).

Adrenocortical tissue of several vertebrate species was incubated with [4-^{14}C] corticosterone. Chromatographic examination of the radioactive steroids produced by interrenal tissue of a marine teleost, the Atlantic herring, *Clupea harengus harengus*, indicated no 1α-hydroxycorticosterone but did suggest that aldosterone and 18-hydroxycorticosterone had been formed. Results of this first experiment are shown in Fig. 1: it is a tracing of the radiochromatogram obtained from extracts of the incubation of [4-^{14}C] corticosterone with the head kidney and cardinal veins (the site of adrenocortical tissue in teleosts) of small immature herring and with posterior kidney

J. Fish. Res. Bd. Canada, 25(2): 431–435, 1968.
Printed in Canada.

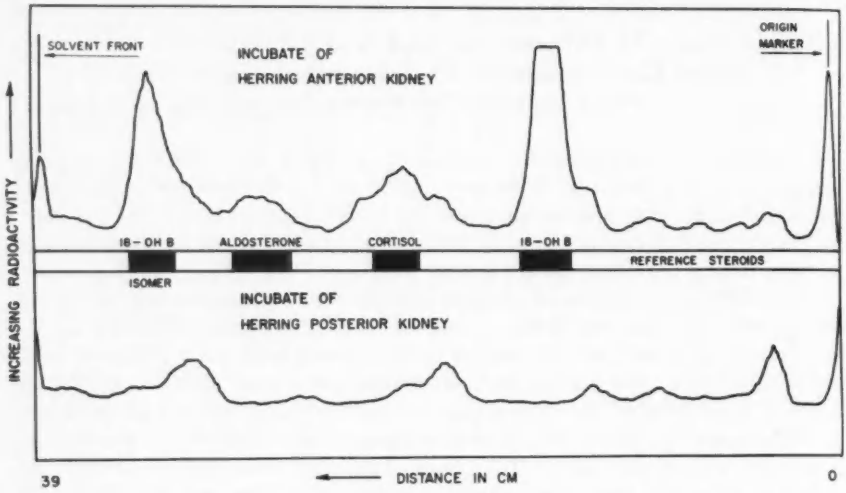

FIG. 1. Scan of radioactivity on paper chromatograms developed for 12 hr with Bush B_5 solvent system.

tissue included as a control. The experiment with herring tissue was repeated in order to establish the identity of the compounds which behaved chromatographically as aldosterone and 18-hydroxycorticosterone.

Herring caught in local waters were transported live to the laboratory. Within 6 hr of capture the fish were killed and the head kidney with the anterior part of the post-cardinal vein was removed. Thus, 1.04 g of tissue was collected from five fish; in this experiment, three of the fish were mature adults, two male and one female. The tissue was incubated at 26 C in 10 ml Tris-buffered medium with added NADPH-generating factors. Details of incubation procedures as well as methods of extraction and isolation of steroids, chromatography, detection and measurement of radioactivity were described previously (Idler and Truscott, 1967). The substrate, 25.2 μc [1,2-^{3}H] corticosterone (6 μg/mc), was purified by paper chromatography and added to the tissue in 0.1 ml propylene glycol. The incubation was stopped after 5 hr by the addition of acetone. A final dichloromethane extract of the incubated herring tissue was prepared as described by Psychoyos et al. (1966). Radioactivity measurements of this solution indicated that only 29% of the initial radioactivity of the substrate was recovered in the organic phase. This neutral steroid fraction was examined further.

Approximately 100 μg authentic aldosterone, 18-hydroxycorticosterone, and cortisol, as a marker, were added to the total extract. Paper chromatography in benzene:50% methanol, Bush B_5 system, again separated the added steroids and radioactive zones as illustrated in Fig. 1.

[^{3}H] Aldosterone was identified in the aldosterone area. Authentic [4-^{14}C] aldosterone, checked for purity by chromatography as described by the supplier, New England Nuclear Corp., Boston, Mass., was added to the [^{3}H] labelled suspect such that the ^{3}H:^{14}C ratio equalled 14.4:1.00. The mixture of isotopes with carrier aldosterone was chromatographed on thin-layer silica in the following solvent systems: I, ethyl acetate:methanol (20:1); II, chloroform:methanol:water (90:10:1); III, ethyl acetate:cyclohexane (10:1); and IV, ethyl acetate:cyclohexane:ethanol (45:45:10). The steroids were further purified by paper chromatography in benzene:hexane:methanol:water (3:1:2:1) and in system E_2B, iso-octane:*t*-butanol:water (50:25:45). When the ^{3}H:^{14}C ratio reached 0.914:1.00, the [^{3}H] labelled steroid could not be separated further from [^{14}C] aldosterone by chromatography. After the final E_2B chromatogram, the eluate was diluted with 11 mg crystalline aldosterone. The preparation was then recrystallized until the isotope ratio was constant and the ratio of the crystals equalled the ratio of the mother liquor. Crystallization data are listed in Table I. From these data 6.5% of the radioactivity in the aldosterone area (Fig. 1) was [^{3}H] aldosterone.

TABLE I. Identification of [^{3}H] aldosterone produced by herring interrenal tissue from [^{3}H] corticosterone with authentic [^{14}C] aldosterone by serial crystallization.

Crystallization no.	Radioactivity (*dpm*)					
	Crystals			Mother liquor		
	^{3}H	^{14}C	Ratio	^{3}H	^{14}C	Ratio
1	285	324	0.880	1269	1389	0.914
2	395	460	0.859	1318	1490	0.885
3	505	576	0.878	554	644	0.860
4	560	636	0.881	488	553	0.883

Since [^{14}C] 18-hydroxycorticosterone is not available from commercial sources, it was prepared biosynthetically. Adrenal tissue from toads, *Bufo marinus*, was incubated with chromatographically pure [4-^{14}C] corticosterone according to the method described for the biosynthesis of 18-hydroxycorticosterone by adrenal tissue of American bullfrogs (Ulick and Vetter, 1962). The radioactive product corresponding in mobility to authentic 18-hydroxycorticosterone on the initial paper chromatogram (Bush B_5) was further purified by chromatography on silica in solvent system II and on paper in the E_2B system. An aliquot of this preparation was then added to the [^{3}H] labelled 18-hydroxycorticosterone area, containing carrier, of the herring incubate. The initial ^{3}H:^{14}C ratio was 13.9:1.00. Isopolarity of the [^{3}H] and [^{14}C] labelled steroids was established by thin-layer and paper chromatography as listed in Table II. The eluate of the last chromatogram was divided

TABLE II. Homogeneity through chromatography and derivative for mationof [^{3}H]18-hydroxycorticosterone (18-OH B) isolated from an incubate of herring interrenal tissue, and [^{14}C]18-OH B biosynthesized by amphibian adrenal tissue. Authentic *dl*-18-OH B was added as carrier.

Chromatographic system	Compound	^{3}H:^{14}C isotope ratio
TLC,[a] chloroform:methanol:water (90:10:1)	18-OH B	5.98
TLC, ethyl acetate:methanol (20:1)	18-OH B	5.03
	(First) isomer of 18-OH B	4.66
	(Second) isomer	4.77
Paper, E_2B	18-OH B	4.78
TLC, ethyl acetate:chloroform:methanol (90:10:1)	18-OH B acetate	4.72
TLC, ethyl acetate:benzene (10:1)	18-OH B acetate	4.80
Paper, Bush B_3	18-OH B acetate	4.72
TLC, ethyl acetate:benzene (10:1)	(20,18)-γ-lactone 18-OH B	4.68
Paper, Bush B_3	(20,18)-γ-lactone	4.64

[a]TLC = thin-layer chromatography.

into two portions. One portion was acetylated with acetic anhydride in pyridine and the second portion was oxydized with periodic acid to the lactone of 11β,18-dihydroxy-3-keto-4-etienic acid (Raman et al., 1964). Isotope ratios of these derivatives are included in Table II. Thus, 29% of radioactivity in the 18-hydroxycorticosterone area (Fig. 1) was [^{3}H]18-hydroxycorticosterone. 18-Hydroxycorticosterone decomposes in a characteristic fashion during chromatography and can exist in at least three different tautomeric forms (Sandor and Lanthier, 1963). In this study, when the less polar isomers could be isolated, the isotope ratio was determined and is included in Table II.

The present study establishes that herring interrenal possesses the enzyme systems necessary to convert corticosterone to aldosterone. It remains to be determined if aldosterone is produced in vivo at a rate sufficient to reach biologically active levels in body fluids. The extent to which 17-deoxycorticoids would be available in vivo as aldosterone precursors in herring is unknown. Caution must be exercised in reasoning by analogy with other teleosts, but in the absence of data on steroids of herring certain facts may be worth noting. Corticosterone has been identified in the blood of sockeye salmon but the level was extremely low (e.g., 0.05 μg/100 ml) and 11-deoxycorticosterone was not detected (Idler et al., 1959). This is in marked contrast to the substantial levels of various 17α-hydroxylated steroids (Schmidt and Idler, 1962). It is of great interest that corticosterone is converted to cortisol by interrenal of an eel (*Anguilla anguilla*) (Sandor et al., 1966) since this unexpected pathway, coupled with a strong corticosteroid 17-hydroxylating system demonstrated for several teleosts, would presumably compete for potential aldosterone precursors.

We thank Dr R. Neher, Ciba A.G., Basle, for a gift of *dl*-18-hydroxycorticosterone.

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Received for publication January 13, 1968.

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